Neurobehavioral Effects of Neonatal Methylmercury Exposure: Impacts on Perseveration and Learning

by

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A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
May 7, 2022

Keywords: Neonatal Development; Methylmercury; Behavior; Dopamine Neurotransmission

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Abstract

Neural changes occurring during the neonatal period in rodents are akin to those seen in humans during the third trimester of pregnancy. The neonatal period is a developmental period during which the monoamine systems develop so they are sensitive to external disruption. Methylmercury (MeHg), an environmental contaminant, disrupts neurobiology and behavior following exposure during various developmental periods but the neonatal period has not been modeled, partly because breast milk is a poor source of bioavailable methylmercury. To examine this developmental period, male Long-Evans rats were exposed to 0, 80, or 350 µg/kg/day MeHgCl from postnatal days 1 to 10, the rodent neonatal period. As adults, behavioral flexibility, attention, memory and expression of the dopamine transporter, DAT, in these rats was assessed. Rats exhibited changes in behavioral flexibility assessed in a spatial discrimination reversal procedure. Those rats exposed to 350 µg/kg/day MeHgCl more quickly transitioned in responding on the new lever on the second reversal and more slowly made this transition on the third reversal. Rats exposed to this dose also acquired responding in the absence of a signal more slowly, and to a lesser degree, during acquisition of the attention/memory procedure but neither attention nor memory were affected once the task was acquired. Finally, DAT expression in the striatum, PFC, and hippocampus was unchanged in these adult rats. The results of this study replicate the trend of findings seen with exposure during gestation or during adolescence.

Acknowledgments

I would like to thank Dr. Newland and Dr. Reed for their help and guidance throughout this research project. I would also like to extend a thank you to the members of my dissertation committee for their guidance and direction. I would also like to thank Dr. Bhattacharya for his guidance and mentoring on the performance of western blots. Finally, I would like to extend thanks to David Haste, Gabrielle Pollard, Olivia Cobb, Carleigh Morrow, Caroline Carter, Miles Wiley, Kawsar Chowdhury, Kelli McDonald, and Warren Smith for their training and help with data collection. This project was possible thanks to funding from the National Science Foundation Graduate Research Fellowship (DGE-1937964) and the Sydney W. and Janet R. Bijou Grant from the Society for the Advancement of Behavior Analysis International.

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MeHg	Methylmercury
ADHD	Attention-Deficit/Hyperactivity Disorder
DAT	Dopamine Transporter
SERT	Serotonin Transporter
GABA	Gamma Aminobutyric Acid
NMDA	N-Methyl-D-Aspartate
DNMTS	Delayed Non-Match to Sample
LAT	Large Amino Acid Transporter
DRH	Delayed Reinforcement of High Rates
MAO	Monoamine Oxidase
PND	Postnatal Day
DOPAC	3’4-Dihydroxyphenylacetic Acid
ITI	Intertrial Interval
FR	Fixed Ratio
SDR	Spatial Discrimination Reversal
OD	Original Discrimination
R1-3	Reversal 1-3
TO	Timeout
PFC	Prefrontal Cortex

RIPA	Radioimmunoprecipitation Assay Buffer
PVDF	Polyvinylidene Difluoride
TBST	Tris-Buffered Saline with Tween80
NFDM	Nonfat Dry Milk
IgG	Immunoglobulin G
ECL	Enhanced Chemiluminescence
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
CR	Correct Rejection
FA	False Alarm
P(Hit)	Hit Rate
P(FA)	False Alarm Rate
LME	Linear Mixed Effects

Chapter 1
Literature Review

Introduction

Methylmercury on Environment and Behavior

Early life exposure to the environmental neurotoxicant, methylmercury (MeHg), produces severe deficits in neural development, impairments in functions related to the prefrontal cortex, and motor deficits (Belém-Filho et al., 2018; Boucher et al., 2012; Grandjean et al., 2014; Chandran et al., 2019; Reed et al., 2006; Reed & Newland, 2009; Tian et al., 2016). Human MeHg exposure occurs via the consumption of contaminated marine fish, especially long-lived predators that occupy a high tropic level, such as shark, tilefish, tuna, swordfish, king mackerel, and many other species (Barone et al., 2021; Groth, 2010), or consumption of rice from contaminated patties (Feng et al., 2021; Li et al., 2010). Impacts of developmental exposure to MeHg are seen in children born to mothers who consume high amounts of predatory fish in the diet during gestation (Bosch et al., 2016; Castaño et al., 2015; Clarkson, 1992), or who live in regions that consume rice from contaminated rice patties, such as in farmlands in China located near mining operations (Feng et al., 2021; Feng et al., 2008; Zhao et al., 2019). In animal models, MeHg is distributed throughout the body via the blood before accumulating in brain, muscle tissue, liver, kidneys, pituitary, and other organs (Bosch et al., 2016; Peng et al., 2016; Rand et al., 2020). The amount accumulated depends on the route of exposure, the dose of exposure, and the species. MeHg readily passes through physiological membranes (Roos et al., 2010; Spiller, 2018) and is capable of passing through both the blood brain barrier, resulting in a large accumulation of MeHg in areas such as the cerebellum and cortex (Bosch et al., 2016;

Oliveira et al., 2018; Roos et al., 2010), and the placental barrier, resulting in a high risk of fetal toxicity (Grandjean et al., 1997; Newland et al., 2008b; Sakamoto et al., 2018).

Animal models of prenatal exposure to MeHg, accomplished by way of maternal diet or drinking water, reveal deficits in motor function, behavioral flexibility, response inhibition, learning, and choice in the offspring (Montgomery et al., 2008; Newland et al., 2004, 2013; Onishchenko et al., 2007; Reed et al., 2006). Many such deficits are mirrored in humans, with children and adolescents exposed to MeHg during gestation showing impairments in motor ability, learning, memory, attention, language, and behavioral flexibility (Boucher, Jacobson, et al., 2012; Debes et al., 2006; Grandjean et al., 1997; Santos-Lima et al., 2020). The prenatal period is not the only developmental stage sensitive to MeHg's neurotoxic effects. Exposure occurring during the late postnatal or adolescent periods in rodents has also been shown to be sensitive to MeHg's effects with impairment in motor ability, perseveration, and choice, being most consistently observed (Belém-Filho et al., 2018; Boomhower & Newland, 2016, 2017; Oliveira et al., 2018; Kendricks et al., under review; Sakamoto et al., 2004; Tian et al., 2016).

Perseveration, or the inability of behavior to change when the contingencies that control it are changed, has been a consistent marker for MeHg toxicity in animal models following both prenatal and adolescent exposure. Perseveration has also been associated, to some degree, with concurrent exposure in humans. Perseverative responding is observed in an array of measures designed to determine the flexibility of behavior, including attentional set shifting, discrimination reversal (spatial and visual), and response inhibition. In humans, perseverative behavior is most often detected using cognitive tests designed to measure loss of function associated with neurodevelopmental disorders (such as the CANTAB). Perseverative errors are sometimes associated with elevated hair MeHg content in young adults living in the Seychelles

(van Wijngaarden et al., 2017) suggesting a link with more recent exposure but not gestational exposure. There was no link between maternal hair Hg collected during pregnancy and perseverative errors when children were 17 or when they were between 22 and 24 years of age in this population (Davidson et al., 2011; van Wijngaarden et al., 2017), suggesting that the perseveration was unrelated to prenatal exposure.

The occurrence of perseverative errors has been seen more consistently in animal models. Elevated errors in a spatial discrimination task were observed in adult rats following prenatal exposure (Paletz et al., 2006; Reed et al., 2006) and adult mice following exposure during adolescence (Boomhower & Newland, 2017). A similar trend was observed in a non-cued spatial alternation task for rats exposed during gestation (Widholm et al., 2004). Perseverative behavior linked to developmental MeHg exposure persists throughout the lifespan with prenatal exposure impairing performance in the spatial discrimination task both in adult and geriatric rats (Paletz et al., 2007). Notably, while perseverative errors arose in animal models following both pre- and post-natal exposure, this has not been the case in humans. The reason could be related to the doses of MeHg observed in these studies, concurrent exposures to other compounds, such as fatty acids, that may ameliorate these effects in humans (Strain et al., 2008), or a relative insensitivity of the tests used to assess perseveration in humans.

The observed perseverative behavior in humans and rodents following exposure to MeHg during various developmental stages may be related to impaired choice or reinforcement processing. Evidence for this conclusion comes both in the alteration of the acquisition of choice observed in non-human primates (Newland et al., 1994) and adult rats (Newland et al., 2004) following prenatal exposure as well as the impaired reinforcement processing observed in adult mice following exposure to MeHg during adolescence (Boomhower & Newland, 2019a, 2019b).

While perseverative behaviors are readily observed in models of early MeHg neurotoxicity, other markers of higher function do not show such consistent results. Remembering, for example, is impaired by several heavy metal toxicants, including arsenic, cadmium, and lead. While some animal models of prenatal and/or postnatal exposure to MeHg show some degree of impairment in remembering (Albores-Garcia et al., 2016; Sakamoto et al., 2004; Tian et al., 2016), this is not always the case (Gilbert et al., 1993; Goulet et al., 2003; Kendricks, Boomhower, & Newland, 2020), and where effects have been reported in animal studies, they appear only following very high exposure levels. This inconsistency is also true for epidemiological studies. Early-life MeHg toxicity, measured either by maternal hair/blood during pregnancy or by hair taken from children at the time of behavioral assessment, was associated with impaired remembering in some reports of children living in the Faroe Islands and the Seychelles Islands, and both children and adolescents living near the Madeira River in Brazil (Davidson et al., 2006; Grandjean et al., 1997, 2014; Santos-Lima et al., 2020). The children observed by Davidson and colleagues (2006) showed minor impairment in memory when they were 5 years of age, but no such relation was observed when these children were much younger, between 9 and 30 months of age, nor when they were much older, at 22-24 years of age (Davidson et al., 2008; van Wijngaarden et al., 2017) and the investigators believe this does not represent a reliable deficit. Similarly, in other studies these effects in humans appear to vary depending on many different factors, two of which include the developmental window of exposure and when assessment occurred in relation to exposure. Children living in the communities of French Guiana had no deficits in memory associated with prenatal exposure but did show deficits in short-term memory associated with their hair MeHg levels, a marker of recent exposure (Cordier et al., 2002). This variability makes conclusions about the impact of

MeHg on memory, if they exist, ambiguous and their presence is likely linked to when exposure occurred and the dose of exposure.

The inconsistent effects of MeHg on memory resemble the absence of a consistent association between MeHg and altered attentional processes. In some human studies, MeHg is shown to be associated with higher prevalence of ADHD diagnosis or ADHD-like behavior (Boucher, Jacobson, et al., 2012; Cheuk & Wong, 2006; Sagiv et al., 2012), but others fail to identify such a relation (Ha et al., 2009; Kim et al., 2013; Polańska et al., 2013). The inconsistency is not because of difficulties in detecting such a relation as there are clear associations between heavy metals and ADHD that are expected, especially, lead. The lack of clear association between MeHg and ADHD diagnosis may lead to a suggestion that MeHg does not interact with behaviors typical of this disorder, namely inattention, hyperactivity, and impulsivity, however, this may not necessarily be the case. Prenatal exposure to MeHg has been shown to affect sustained attention in human populations (Boucher, Jacobson, et al., 2012; Grandjean et al., 1997) but not impulsivity (Boucher, Burden, et al., 2012). Interestingly, however, adolescent MeHg exposure appeared to improve impulsive choice in animal models (Boomhower & Newland, 2016). Importantly, while prenatal exposure appears to impair attention, this is not the case for postnatal exposure as no clear association has been shown between inattention and concurrent blood MeHg levels in humans (Boucher, Jacobson, et al., 2012) or in rodents exposed to MeHg during adolescence (Kendricks, Boomhower, Arnold, et al., 2020; Kendricks & Newland, 2021). While it would appear that sustained attention in both humans and non-humans is sensitive to prenatal, but not late postnatal or adolescent, MeHg toxicity, this conclusion cannot be drawn yet due to the lack of report of impaired attention following prenatal exposure in animal models.

In summary, MeHg exposure occurring during either the prenatal or adolescent periods has lifelong effects. Prenatal exposure in both humans and nonhumans affects both behavioral flexibility and sustained attention and may potentially affect memory. On the other hand, adolescence is less sensitive, though modest impairment in perseveration, and some reports of impairment in memory, have been reported. This sensitivity to developmental periods is of interest as it highlights the importance of age and developmental stage in understanding the toxicity of MeHg.

Animal models of developmental MeHg toxicity are accomplished by exposing pregnant animals and observing pups later in life or by exposing animals during some period of postnatal development and assessing changes in behavior during or after exposure. Both of these approaches provide information about MeHg's developmental toxicity.

A key period of development is often overlooked. The neonatal period in rodents encompasses the first 10 days after birth but the physiological, neurochemical, and neuroanatomical changes occurring during this early postnatal period are akin to those changes occurring during the third trimester of human pregnancy (Semple et al., 2013). Most studies of prenatal exposure in rodents extend exposure out until weaning by providing MeHg in the maternal food or drinking water with the presumption that pups will continue to be exposed via the mother's milk during lactation. This is not the case, however. Mercury in breast milk is largely in the form of water soluble $HgCl_2$ and is not bioavailable (Newland & Reile, 1999; Newland et al., 2008; Sakamoto et al., 2002). Both Newland and Reile (1999) and Sakamoto and colleagues (2002) showed that blood MeHg levels of pups decreases substantially after birth despite their nursing from mothers whose exposure continues. This dramatic decrease is because mercury in breast milk is in the form of mercuric chloride which is negligibly bioavailable

(Newland et al., 2009). Because of this, studies that extend maternal exposure throughout gestation and early postnatal development fail to include the period of development synonymous with the third trimester of human gestation. Because developmental periods are differentially sensitive to MeHg's effects, the absence of reports of exposure during this development window in rodents is a severe drawback in drawing comparisons between rodent and human models of prenatal MeHg's toxicity.

Neurobiology of Behavior

Perseveration, short-term memory, and sustained attention are all complex processes governed by multiple neurotransmitter systems. These behaviors are closely linked to many other processes making determining their sensitivity to external disruptions, such as MeHg, a tricky task to undertake. In order to understand the sensitivities of these behaviors better, we will discuss briefly the neurobiology of each.

Perseverative behavior, or the persistence of behavior after the contingencies selecting that behavior have changed, is driven by monoaminergic activity in the orbitofrontal cortex, dorsolateral prefrontal cortex, and striatum. Perseverative errors are associated with polymorphisms of the dopamine transporter, DAT (den Ouden et al., 2013). On the other hand, switching rate, or the likelihood an individual will alter responding after encountering punishment of a particular alternative, is associated with expression of polymorphisms of the serotonin transporter, SERT (den Ouden et al., 2013). Dopaminergic mediation of perseverative responding appears to be localized to the striatum. Increased dopamine activity in the striatum is associated with accurate prediction of a stimulus after reversal, an effect that is impacted by the dopamine D_2 agonist, bromocriptine (Cools et al., 2009). Acquisition of reversal tasks is mediated by activity within the striatum as well as in the frontal cortex (Kehagia et al., 2010;

Overman, 2004) with lesions of the orbitofrontal cortex, the anterior cingulate, as well as the dorsolateral prefrontal cortex producing higher numbers of perseverative errors (Brown & Bowman, 2002; Chudasama et al., 2003).

Short-term memory is a complex phenomenon impacted by structures including the prefrontal cortex (Aalto et al., 2005; Cohen et al., 1997; Kane & Engle, 2002) and regions of the medial temporal lobe, the hippocampus and amygdala (Aalto et al., 2005; Bird & Burgess, 2008; von Allmen et al., 2013). Short-term memory is mediated by glutamate. Young adult rats trained on a Morris water maze procedure have longer path lengths and longer latencies to reach the platform following exposure to high doses of memantine and MK801, antagonists of the NMDA receptor (Duda et al., 2016). Further, short-term memory is mediated by acetylcholine. Acute administration of the cholinergic antagonists, scopolamine and mecamylamine, caused disruption in performance in T-maze procedures designed to test working and reference memory. Namely, scopolamine dose-dependently impaired performance in the working memory procedure while mecamylamine dose-dependently impaired performance in the reference memory procedure (Moran, 1993). Further, acute administration of nicotine into the basolateral amygdala facilitates remembering of aversive stimuli in rats, in contrast to the diminished retention of this stimulus following administration of the nicotinic antagonist, mecamylamine (Barros et al., 2005).

Short-term memory is also mediated by monoaminergic activity. Haloperidol, a dopamine antagonist, impairs working memory in young adult male mice when administered during the retention interval, or the time between when the stimulus was presented and when the stimulus was to be recalled (Beatty & Rush, 1983). Further, moderate to high doses of *d*-amphetamine, a drug that stimulates release and inhibits reuptake of dopamine and norepinephrine, and quinpirole, a dopamine D_2/D_3 agonist, impair short-term memory in adult

male rats trained on a delayed non-match to sample task (DNMTS) (Bushnell & Levin, 1993). This was not the case for the D_1 agonist, SKF 38393, the D_1 antagonist, SCH 23390, or the D_2 antagonist, raclopride (Bushnell & Levin, 1993). While dopamine plays a major role in the mediation of processes of short-term memory, it is not the only monoamine to do so. Both norepinephrine and serotonin influence processes of remembering. Rats exposed chronically to moderate doses of either clomipramine, a serotonin reuptake inhibitor, or desipramine, a norepinephrine reuptake inhibitor, showed impaired performance in a radial arm maze procedure as young adults (Burgos et al., 2005).

Attention is largely mediated by activity within the prefrontal cortex, anterior cingulate cortex, and superior parietal cortex (Aalto et al., 2005; Davis et al., 2000; Pardo et al., 1991). Modulation of attentional processes is controlled by activity of acetylcholine, dopamine, and norepinephrine. Sustained attention is differentially affected by dopamine agonists in a baseline-dependent fashion: Impaired attention is improved by *d*-amphetamine while unimpaired attention is either unaffected or is impaired by *d*-amphetamine (Bizot et al., 2015; Sagvolden & Xu, 2008). This is seen in a study performed by Bizot and colleagues (2015) in adolescent rats. Wistar and SHR rats were trained on a two-choice attention task where animals had to track a brief signal light. During training, Wistars performed worse than SHRs, but during testing *d*-amphetamine improved Wistars' accuracy but not SHRs' (Bizot et al., 2015). Sustained attention is also impacted by norepinephrine. Agonists and antagonists of norepinephrine dose-dependently reduce accuracy in a two-choice visual signal detection task (Bushnell et al., 1997). This effect could also be attributed to baseline-dependency with unimpaired attention being hindered by norepinephrine agonists but impaired attention being improved.

Of more importance is the influence of acetylcholine on attentional processes. While it would appear that agonists of acetylcholine impair attention, this is likely also a baseline-dependent effect. Impaired attention is improved by agonists of acetylcholine but when attention is not impaired agonists either reduce accuracy or exert no effect (Bushnell et al., 1997). This is seen in that cholinergic antagonists impair attention (Bushnell et al., 1997), but also in that the loss of cholinergic function (Cherian et al., 2019; Mohler et al., 2001) causes severe impairment in attentional processes. Further evidence of this comes in that attentional impairment caused by cholinergic lesions in the basal forebrain in rodents can be rescued by administration of the positive muscarinic allosteric modulator, TAK-071 (Kucinski et al., 2020).

While perseveration, short-term memory, and attention are all partially mediated by activity within the prefrontal cortex, they are also all partially mediated by activity within the basal ganglia, specifically the striatum. Striatal activity governs goal-directed behavior and dictates reinforcement processing necessary to establish robust patterns of behavior in tasks that assess perseveration (Cools et al., 2009; Izquierdo et al., 2017; Kehagia et al., 2010). The striatum also plays a role in the filtering of information in tasks assessing memory. It is hypothesized that this shunting of information is due to stimulation of dopaminergic neurons in the striatum allowing inhibition of signals communicating the presence of distractors to be transmitted to the prefrontal cortex (McNab & Klingberg, 2008). Finally, the striatum has been implicated in disease models for multiple different psychological disorders, one being attention-deficit/hyperactivity disorder (Cheon et al., 2003; del Campo et al., 2011; Dougherty et al., 1999) suggesting a possible role of the striatum in the mediation of attentional processes. Importantly, both the prefrontal cortex and the striatum are hubs for monoaminergic neurotransmission (Coyle

& Molliver, 1977; Larsen et al., 2020), especially dopamine, which has been shown to influence these behaviors, and to be influenced by MeHg (Kalisch & Racz, 1996).

Neurobiology of Methylmercury

MeHg is transported across physiological barriers, including the blood-brain barrier and the placental barrier. This transport is facilitated by binding of MeHg to either sulfhydryl or thiol moieties. Binding of MeHg to the sulfhydryl moiety, cysteine, results in the formation of a cysteine-MeHg complex that resembles the amino acid, methionine (Aschner, 1989; Clarkson & Magos, 2006; Spiller, 2018). Because of this similarity, MeHg complexes can be readily transported across physiological barriers by a neutral amino acid carrier. While MeHg can be taken into the liver and kidney, facilitating its excretion (Bosch et al., 2016; Roos et al., 2010), binding of MeHg to cysteine allows it to pass through the blood brain barrier, most likely through the function of the large amino acid transporter 2 (LAT2) (Balthasar et al., 2017; Clarkson & Magos, 2006; Spiller, 2018). It is hypothesized that part of the toxicity attributed to MeHg is the reduced accessibility of the antioxidant, selenium, and therefore selenoproteins, due to the binding of selenium to Hg (Spiller, 2018). The reduced availability of selenoproteins may have implications on a large host of physiological systems, such as with alterations in neurotransmitter release, increased risk of oxidative damage to cells due to a decrease in selenium's antioxidant effects, and many others.

Acute and chronic MeHg exposure affects growth, motor function, and behavior, depending on when exposure occurred, the dose of exposure, and when testing occurred in relation to exposure. We will discuss here the implications of both acute and chronic exposure to MeHg on sustained attention, short-term memory, and perseveration. To review, sustained attention is mediated by the anterior cingulate, prefrontal cortex, and premotor areas (Aalto et al.,

2005; Fortenbaugh et al., 2017); short-term memory is mediated by connections among the prefrontal cortex, basal ganglia, and areas within the medial temporal lobe, including the hippocampus (Aalto et al., 2005; Bird & Burgess, 2008; McNab & Klingberg, 2008); and finally, perseveration is mediated by the striatum, orbitofrontal cortex, and prefrontal cortex (Kehagia et al., 2010). Together, these suggest that MeHg may interact with these systems due to the wide array of targetable areas. But this raises the concern of what aspects of these behaviors may be targeted by MeHg toxicity and are these behaviors differentially sensitive depending on when such exposure to MeHg occurs.

MeHg disrupts the monoamines, glutamate, and GABA. Adult rats administered MeHg acutely via dialysis exhibit a spike in striatal dopamine that is reduced in the presence of antagonists of the glutamate NMDA receptor (Faro et al., 2002b). This suggests that striatal release of dopamine is mediated by glutamatergic signaling. Further, MeHg contributes to the excitotoxic effects of glutamate. MeHg accumulates in astrocytes where it impairs reuptake of glutamate resulting in the accumulation of glutamate in the synapse which may result in excitotoxicity and cell death (Aschner et al., 2000; Castoldi et al., 2001). MeHg's influence on GABA is likely associated with neuromotor deficits observed following exposure to high concentrations of MeHg as MeHg accumulates in the cerebellum and motor areas, regions rich in GABA, resulting in loss of fine motor control and motor learning (Atchison, 2005; Oliveira et al., 2018; Sakamoto et al., 2004; Santana et al., 2019). Rats exposed to high doses of MeHg during gestation also show heightened sensitivity to the $GABA_A$ agonist, pentobarbital, when tested on a differential reinforcement of high rates (DRH) procedure to assess reinforcement sensitivity and motor function in adulthood (Rasmussen & Newland, 2001). To strengthen this

evidence, it was reported that GABA sensitivity was dependent on MeHg dose, pentobarbital dose, and the developmental period.

In animal models, acute administration of relatively high doses of MeHg into the striatum increases release of dopamine and inhibits the action of DAT in adult rats (Faro et al., 1998, 2000, 2002a). This effect resembles that of *d*-amphetamine. Prenatal exposure to a high concentration of MeHg is also shown to impair activity of the enzyme, monoamine oxidase B (MAO-B), in the cerebellum of weanling male rodents (Castoldi et al., 2006), which may be associated with changes in dopamine signaling coming from nuclei of the striatum or other regions. MeHg's effects are not limited to dopamine activity, however, as MeHg also inhibits reuptake of norepinephrine and serotonin, while increasing release of these neurotransmitters, in synaptosomes of the occipital cortex and hypothalamus, respectively (Komulainen & Tuomisto, 1981). Further, acute MeHg decreases whole-brain serotonin content in fish exposed in adulthood with this reduction accompanied by higher anxiety-indicative behavior, namely light avoidance and higher incidence of erratic swim patterns and bottom-tank dwelling in a novel environment (Maximino et al., 2011). MeHg does not, however, appear to influence norepinephrine activity in other brain regions, such as the prefrontal cortex and striatum. This is evidenced by a lack of change in behavior mediated by these regions, namely reinforcement processing, memory, and response inhibition, in the presence of agonists of norepinephrine despite a pronounced change in these behaviors in the presence of a dopamine agonist (Boomhower & Newland, 2019b; Kendricks & Newland, 2021; Reed & Newland, 2009). Importantly, this was true regardless of when exposure occurred, either prenatally or during adolescence.

These neurotransmitter systems each play a large role in behavior, as noted earlier. Sustained attention is largely mediated by activity of acetylcholine, dopamine, and norepinephrine (Bushnell et al., 1997; Sagvolden & Xu, 2008). Perseveration is largely mediated by activity of dopamine, norepinephrine, and serotonin (Izquierdo et al., 2017; Kehagia et al., 2010). Finally, memory is largely mediated by dopamine, norepinephrine, serotonin, acetylcholine, and glutamate (Barros et al., 2005; Burgos et al., 2005; Bushnell & Levin, 1993; Duda et al., 2016). It is unsurprising, then, that each of these behaviors are shown to be impacted by developmental exposure to MeHg, whether such exposure occurred prenatally or postnatally, due to the influence of MeHg on each of these neurotransmitter systems. However, while MeHg is shown to influence each of these behaviors, with such influence likely associated with changes in neurotransmission, it is important to consider the nuance of these interactions. As discussed, MeHg's influence on these behaviors is dependent both on the dose of MeHg and when exposure occurred. This is important when attempting to understand MeHg's neurotoxic effects as changes in these neurotransmitter systems during different stages in development may mark the differences in relative sensitivity of these time periods to the neurotoxic effects of MeHg.

Developmental Sensitivity: Neonatal Methylmercury

Exposure to MeHg either during gestation or during adolescence causes severe changes in neurobiology and behavior with these changes differing depending on the developmental period. The adolescent period appears to be less sensitive to MeHg's toxicity than the prenatal period. Rodents trained on a spatial discrimination reversal (SDR) task, designed to measure perseverative behavior, performed differently depending on whether exposure to MeHg occurred during gestation or during adolescence. Rats exposed to a high dose of MeHg during gestation showed impaired performance on the first reversal of the SDR task in adulthood, seen by a larger

number of required sessions to achieve criterion accuracy for the next reversal (Paletz et al., 2007; Reed et al., 2006). While mice exposed to a high dose of MeHg during adolescence, the same high dose used by Paletz and colleagues (2007), also showed impairment in this SDR procedure, this effect was more subtle. Exposed mice in this study took longer to achieve criterion accuracy on their second reversal but with a comparatively smaller number of required sessions to achieve this criterion when compared to the rats on their first reversal (Boomhower & Newland, 2017). These differences highlight how MeHg's toxicity is contingent on when exposure occurred. However, they also illustrate that the dose of exposure is important as deficits in rats on this SDR procedure only arose for those animals exposed to the high, but not the low, dose of MeHg during gestation (Paletz et al., 2007).

The prenatal period in humans is highly sensitive to neurotoxicant exposure but rodent models of prenatal exposure often fail to encompass the entire span of neural development associated with human gestation. As stated previously, the first 10 days after birth for rodents is akin to the third trimester of pregnancy in humans (Semple et al., 2013), but exposure models using rodents fail to encompass this range because they rely on exposure via breast milk, which does not have bioavailable MeHg (Newland et al., 2008a; Newland & Reile, 1999). Claims of exposure occurring during the neonatal period do exist, however these reports are often confounded by exposure occurring after this period, specifically by exposure occurring throughout the neonatal, late postnatal, and early adolescent periods (Sakamoto et al., 2004; Tian et al., 2016). Further, their interpretation is often limited by the use of very high doses, above that used to model environmental exposures.

Despite the limitations noted, there is some evidence that the postnatal period is sensitive to MeHg. First, MeHg induces a dose-dependent increase in release of dopamine from striatal

synaptosomes of rats from postnatal day (PND) 7-21, with the greatest impact of MeHg observed in synaptosomes from rats at PND 7 (Dreiem et al., 2009). Second, oral exposure to MeHg in male rats from PND 1-30 produces severe motor and cognitive deficits and deterioration of cerebellar and striatal neurons in young adolescent rats (Sakamoto et al., 2004). Finally, Tian and colleagues (2016) exposed rats, *i.p.*, to moderate doses of MeHg from PND 5 through approximately PND 33. Similar to that reported by Sakamoto and colleagues (2004), rats in this study showed severe cognitive deficit and deterioration of hippocampal neurons (Tian et al., 2016). While it is clear that the postnatal period is sensitive to MeHg toxicity, because these studies extend exposure out until the early adolescent period, it is indeterminate if deficits observed in these studies is related to exposure occurring during the neonatal, late postnatal, or early adolescent periods.

Development from birth through adolescence for rodents is rich in maturation of neurotransmitter systems, including that of the monoamines. Concentrations of the D1, D2, and D4 dopamine receptors in the rat basal ganglia rise early in development, between PND 7-28, before falling off through adolescence (Tarazi & Baldessarini, 2000). Densities of these receptors steadily increase in the frontal cortex, entorhinal cortex, and hippocampus from PND 7 through PND 60 (Tarazi & Baldessarini, 2000). A similar increase is also observed in both the dopamine transporter, DAT, and the serotonin transporter, SERT, in the basal ganglia (Tarazi et al., 1998). Further, Connell and colleagues (2004) showed sexual dimorphism in levels of dopamine and serotonin in mice from postnatal day (PND) 3 to 5 and a decline in serotonin across all mice from PND 5 to 14. The abundance of changes in monoamine systems gives rise to differential sensitivity of rodents during the postnatal period. As an example, altered monoamine neurotransmission was observed in rats following exposure to 5 or 7.5 mg/kg methamphetamine,

a dopamine agonist, from approximately PND 11-20. These rats displayed reduced neostriatal concentrations of dopamine and norepinephrine at the end of the exposure period with an exacerbated change in norepinephrine in those rats exposed to methamphetamine and housed in a stressful environment such as a deprived housing cage (Jablonski et al., 2017). These studies reveal that the postnatal period is essential to the development of monoamine systems and, thus, is highly sensitive to external disruptions. Further, it shows that neural development during the postnatal development is in a state of flux so ascertaining whether deficits arising following exposure during this period are unique to sensitivity of any one developmental stage is not possible. Therefore, in order to determine if the neonatal period is uniquely sensitive to exposure it must be isolated from exposure during other developmental periods.

Purpose

The proposed study was designed to isolate the sensitivity of the neonatal period and compare the behavioral and neurochemical changes resultant of exposure to MeHg during this period to those observed previously following exposure during other developmental stages, such as the prenatal, late postnatal, and adolescent periods. In order to accomplish this, male rats were exposed to approximately 0, 80, or 350 µg/kg/day Hg from PND 1 to 10. These doses closely resembled those previously used in our lab to examine MeHg's developmental neurotoxicity during the gestational and adolescent periods.

As adults, rats' behavioral flexibility was assessed using a SDR procedure (Boomhower & Newland, 2017; Paletz et al., 2007; Reed et al., 2006) and sustained attention and short-term remembering were assessed using a two-choice visual signal detection task (Kendricks, Boomhower, Arnold, et al., 2020; Kendricks & Newland, 2021). These are behavioral functions that are influenced by monoamine neurotransmission and have been shown to be impacted by

MeHg following exposure during other developmental periods. This study focused on MeHg-induced changes in dopamine neurotransmission as these behaviors have all been shown to be somewhat influenced by dopamine and MeHg is shown to greatly impact dopamine systems following developmental exposure. In order to determine if any observed changes in behavior in these animals was related to changes in dopamine neurotransmission, the density of DAT within the prefrontal cortex, striatum, and hippocampus was also assessed.

References

Aalto, S., Brück, A., Laine, M., Någren, K., & Rinne, J. O. (2005). Frontal and temporal dopamine release during working memory and attention tasks in healthy humans: A positron emission tomography study using the high-affinity dopamine D2 receptor ligand [11C]FLB 457. *Journal of Neuroscience*, *25*(10), 2471–2477. https://doi.org/10.1523/JNEUROSCI.2097-04.2005

Albores-Garcia, D., Acosta-Saavedra, L. C., Hernandez, A. J., Loera, M. J., & Calderón-Aranda, E. S. (2016). Early developmental low-dose methylmercury exposure alters learning and memory in periadolescent but not young adult rats. *BioMed Research International*, *2016*. https://doi.org/10.1155/2016/6532108

Aschner, M. (1989). Brain, kidney, and liver 203Hg-Methyl mercury uptake in the rat: Relationship to the neural amino acid carrier. *Pharmacology & Toxicology*, *65*, 17–20.

Aschner, M., Yao, C. P., Allen, J. W., & Tan, K. H. (2000). Methylmercury alters glutamate transport in astrocytes. *Neurochemistry International*, *37*, 199–206. www.elsevier.com/locate/neuint

Atchison, W. D. (2005). Is chemical neurotransmission altered specifically during methylmercury-induced cerebellar dysfunction? *Trends in Pharmacological Sciences*, *26*(11), 549–557. https://doi.org/10.1016/j.tips.2005.09.008

Balthasar, C., Stangl, H., Widhalm, R., Granitzer, S., Hengstschläger, M., & Gundacker, C. (2017). Methylmercury uptake into bewo cells depends on LAT2-4F2hc, a system l amino acid transporter. *International Journal of Molecular Sciences*, *18*(8). https://doi.org/10.3390/ijms18081730

Barone, G., Storelli, A., Meleleo, D., Dambrosio, A., Garofalo, R., Busco, A., & Storelli, M. M. (2021). Levels of mercury, methylmercury and selenium in fish: Insights into children food safety. *Toxics*, *9*(2), 1–14. https://doi.org/10.3390/toxics9020039

Barros, D. M., Ramirez, M. R., & Izquierdo, I. (2005). Modulation of working, short- and long-term memory by nicotinic receptors in the basolateral amygdala in rats. *Neurobiology of Learning and Memory*, *83*(2), 113–118. https://doi.org/10.1016/j.nlm.2004.10.001

Beatty, W. W., & Rush, J. R. (1983). Spatial working memory in rats: Effects of monoaminergic antagonists. *Pharmacology Biochemistry and Behavior*, *18*(1), 7–12. https://doi.org/10.1016/0091-3057(83)90139-9

Belém-Filho, I. J. A., Ribera, P. C., Nascimento, A. L., Gomes, A. R. Q., Lima, R. R., Crespo-Lopez, M. E., Monteiro, M. C., Fontes-Júnior, E. A., Lima, M. O., & Maia, C. S. F. (2018). Low doses of methylmercury intoxication solely or associated to ethanol binge drinking induce psychiatric-like disorders in adolescent female rats. *Environmental Toxicology and Pharmacology*, *60*, 184–194. https://doi.org/10.1016/j.etap.2018.04.021

Bird, C. M., & Burgess, N. (2008). The hippocampus and memory: Insights from spatial processing. *Nature Reviews Neuroscience*, *9*(3), 182–194. https://doi.org/10.1038/nrn2335

Bizot, J. C., Cogrel, N., Massé, F., Chauvin, V., Brault, L., David, S., & Trovero, F. (2015). D-amphetamine improves attention performance in adolescent Wistar, but not in SHR rats, in a two-choice visual discrimination task. *Psychopharmacology*, *232*, 3269–3286. https://doi.org/10.1007/s00213-015-3974-2

Boomhower, S. R., & Newland, M. C. (2016). Adolescent methylmercury exposure affects choice and delay discounting in mice. *Neurotoxicology*, *57*, 136–144. https://doi.org/10.1016/j.neuro.2016.09.016

Boomhower, S. R., & Newland, M. C. (2017). Effects of adolescent exposure to methylmercury and d-amphetamine on reversal learning and an extradimensional shift in male mice. *Experimental and Clinical Psychopharmacology*, *25*(2), 64–73. https://doi.org/10.1037/pha0000107

Boomhower, S. R., & Newland, M. C. (2019a). Adolescent methylmercury exposure: Behavioral mechanisms and effects of sodium butyrate in mice. *NeuroToxicology*, *70*, 33–40. https://doi.org/10.1016/j.neuro.2018.10.011

Boomhower, S. R., & Newland, M. C. (2019b). d-Amphetamine and methylmercury exposure during adolescence alters sensitivity to monoamine uptake inhibitors in adult mice. *NeuroToxicology*, *72*, 61–73. https://doi.org/10.1016/j.neuro.2019.02.001

Bosch, A. C., O'Neill, B., Sigge, G. O., Kerwath, S. E., & Hoffman, L. C. (2016). Heavy metals in marine fish meat and consumer health: A review. *Journal of the Science of Food and Agriculture*, *96*(1), 32–48. https://doi.org/10.1002/jsfa.7360

Boucher, O., Burden, M. J., Muckle, G., Saint-Amour, D., Ayotte, P., Dewailly, É., Nelson, C. A., Jacobson, S. W., & Jacobson, J. L. (2012). Response inhibition and error monitoring during a visual Go/No-Go task in Inuit children exposed to lead, polychlorinated biphenyls, and methylmercury. *Environmental Health Perspectives*, *120*(4), 608–615. https://doi.org/10.1289/ehp.1103828

Boucher, O., Jacobson, S. W., Plusquellec, P., Dewailly, É., Ayotte, P., Forget-Dubois, N., Jacobson, J. L., & Muckle, G. (2012). Prenatal methylmercury, postnatal lead exposure,

and evidence of attention deficit/hyperactivity disorder among Inuit children in Arctic Québec. *Environmental Health Perspectives*, *120*(10), 1456–1461. https://doi.org/10.1289/ehp.1204976

Brown, V. J., & Bowman, E. M. (2002). Rodent models of prefrontal cortical function. *TRENDS in Neuroscience*, *25*(7), 340–343.

Burgos, H., Mardones, L., Campos, M., Castillo, A., Fernández, V., & Hernández, A. (2005). Chronic treatment with clomipramine and desipramine induces deficit in long-term visuo-spatial memory of rats. *International Journal of Neuroscience*, *115*(1), 47–54. https://doi.org/10.1080/00207450490512641

Bushnell, P. J., & Levin, E. D. (1993). Effects of dopaminergic drugs on working and reference memory in rats. *Pharmacology, Biochemistry and Behavior*, *45*(4), 765–776. https://doi.org/10.1016/0091-3057(93)90119-E

Bushnell, P. J., Oshiro, W. M., & Padnos, B. K. (1997). Detection of visual signals by rats: Effects of chlordiazepoxide and cholinergic and adrenergic drugs on sustained attention. *Psychopharmacology*, *134*(3), 230–241. https://doi.org/10.1007/s002130050446

Castaño, A., Cutanda, F., Esteban, M., Pärt, P., Navarro, C., Gómez, S., Rosado, M., López, A., López, E., Exley, K., Schindler, B. K., Govarts, E., Casteleyn, L., Kolossa-Gehring, M., Fiddicke, U., Koch, H., Angerer, J., Hond, E. Den, Schoeters, G., … Posada, M. (2015). Fish consumption patterns and hair mercury levels in children and their mothers in 17 EU countries. *Environmental Research*, *141*, 58–68. https://doi.org/10.1016/j.envres.2014.10.029

Castoldi, A. F., Blandini, F., Randine, G., Samuele, A., Manzo, L., & Coccini, T. (2006). Brain monoaminergic neurotransmission parameters in weanling rats after perinatal exposure to

methylmercury and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB153). *Brain Research*, *1112*(1), 91–98. https://doi.org/10.1016/j.brainres.2006.07.022

Castoldi, A. F., Coccini, T., Ceccatelli, S., & Manzo, L. (2001). Neurotoxicity and molecular effects of methylmercury. *Brain Research Bulletin*, *55*(2), 197–203. https://doi.org/10.1016/S0361-9230(01)00458-0

Cheon, K. A., Ryu, Y. H., Kim, Y. K., Namkoong, K., Kim, C. H., & Lee, J. D. (2003). Dopamine transporter density in the basal ganglia assessed with [123 I]IPT SPET in children with attention deficit hyperactivity disorder. *European Journal of Nuclear Medicine and Molecular Imaging*, *30*(2), 306–311. https://doi.org/10.1007/s00259-002-1047-3

Cherian, A. K., Tronson, N. C., Parikh, V., Kucinski, A., Blakely, R. D., & Sarter, M. (2019). Repetitive mild concussion in subjects with a vulnerable cholinergic system: Lasting cholinergic-attentional impairments in CHT+/- mice. *Behavioral Neuroscience*, *133*(4), 448–459. https://doi.org/10.1037/bne0000310

Cheuk, D. K. L., & Wong, V. (2006). Attention-deficit hyperactivity disorder and blood mercury level: A case-control study in Chinese children. *Neuropediatrics*, *37*(4), 234–240. https://doi.org/10.1055/s-2006-924577

Chudasama, Y., Passetti, F., Rhodes, S. E. V., Lopian, D., Desai, A., & Robbins, T. W. (2003). Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: Differential effects on selectivity, impulsivity and compulsivity. *Behavioural Brain Research*, *146*(1–2), 105–119. https://doi.org/10.1016/j.bbr.2003.09.020

Clarkson, T. W. (1992). Mercury: Major issues in environmental health. *Environmental Health Perspectives*, *100*, 31–38. https://doi.org/10.1289/ehp.9310031

Clarkson, T. W., & Magos, L. (2006). The toxicology of mercury and its chemical compounds. In *Critical Reviews in Toxicology* (Vol. 36, Issue 8, pp. 609–662). https://doi.org/10.1080/10408440600845619

Cohen, J. D., Perlstein, W. M., Braver, T. S., Nystrom, L. E., Noll, D. C., Jonides, J., & Smith, E. E. (1997). Temporal dynamics of brain activation during working memory task. *Nature*, *386*, 604–608.

Connell, S., Karikari, C., & Hohmann, C. F. (2004). Sex-specific development of cortical monoamine levels in mouse. *Developmental Brain Research*, *151*(1–2), 187–191. https://doi.org/10.1016/j.devbrainres.2004.03.008

Cools, R., Frank, M. J., Gibbs, S. E., Miyakawa, A., Jagust, W., & D'Esposito, M. (2009). Striatal dopamine predicts outcome-specific reversal learning and its sensitivity to dopaminergic drug administration. *Journal of Neuroscience*, *29*(5), 1538–1543. https://doi.org/10.1523/JNEUROSCI.4467-08.2009

Cordier, S., Garel, M., Mandereau, L., Morcel, H., Doineau, P., Gosme-Seguret, S., Josse, D., White, R., & Amiel-Tison, C. (2002). Neurodevelopmental investigations among methylmercury-exposed children in French Guiana. *Environmental Research*, *89*(1), 1–11. https://doi.org/10.1006/enrs.2002.4349

Coyle, J. T., & Molliver, M. E. (1977). Major innervation of the newborn rat cortex by monoaminergic neurons. *Science*, *196*(4288), 444–447. https://doi.org/10.1210/jcem-10-10-1361

Davidson, P. W., Cory-Slechta, D. A., Thurston, S. W., Huang, L. S., Shamlaye, C. F., Gunzler, D., Watson, G., van Wijngaarden, E., Zareba, G., Klein, J. D., Clarkson, T. W., Strain, J. J., & Myers, G. J. (2011). Fish consumption and prenatal methylmercury exposure: Cognitive and behavioral outcomes in the main cohort at 17 years from the Seychelles child development study. *NeuroToxicology*, *32*(6), 711–717. https://doi.org/10.1016/j.neuro.2011.08.003

Davidson, P. W., Myers, G. J., Cox, C., Wilding, G. E., Shamlaye, C. F., Huang, L. S., Cernichiari, E., Sloane-Reeves, J., Palumbo, D., & Clarkson, T. W. (2006). Methylmercury and neurodevelopment: Longitudinal analysis of the Seychelles child development cohort. *Neurotoxicology and Teratology*, *28*(5), 529–535. https://doi.org/10.1016/j.ntt.2006.06.002

Davidson, P. W., Strain, J. J., Myers, G. J., Thurston, S. W., Bonham, M. P., Shamlaye, C. F., Stokes-Riner, A., Wallace, J. M. W., Robson, P. J., Duffy, E. M., Georger, L. A., Sloane-Reeves, J., Cernichiari, E., Canfield, R. L., Cox, C., Huang, L. S., Janciuras, J., & Clarkson, T. W. (2008). Neurodevelopmental effects of maternal nutritional status and exposure to methylmercury from eating fish during pregnancy. *NeuroToxicology*, *29*(5), 767–775. https://doi.org/10.1016/j.neuro.2008.06.001

Davis, K. D., Hutchison, W. D., Lozano, A. M., Tasker, R. R., & Dostrovsky, J. O. (2000). Human Anterior Cingulate Cortex Neurons Modulated by Attention-Demanding Tasks. *J Neurophysiol*, *83*(6), 3575–3577. http://jn.physiology.org/content/83/6/3575

Debes, F., Budtz-Jørgensen, E., Weihe, P., White, R. F., & Grandjean, P. (2006). Impact of prenatal methylmercury exposure on neurobehavioral function at age 14 years.

Neurotoxicology and Teratology, *28*(5), 536–547. https://doi.org/10.1016/j.ntt.2006.02.005

del Campo, N., Chamberlain, S. R., Sahakian, B. J., & Robbins, T. W. (2011). The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. *Biological Psychiatry*, *69*(12), e145–e157. https://doi.org/10.1016/j.biopsych.2011.02.036

den Ouden, H. E. M., Daw, N. D., Fernandez, G., Elshout, J. A., Rijpkema, M., Hoogman, M., Franke, B., & Cools, R. (2013). Dissociable Effects of Dopamine and Serotonin on Reversal Learning. *Neuron*, *80*(4), 1090–1100. https://doi.org/10.1016/j.neuron.2013.08.030

Dougherty, D. D., Bonab, A. A., Spencer, T. J., Rauch, S. L., Madras, B. K., & Fischman, A. J. (1999). Dopamine transporter density in patients with attention deficit hyperactivity disorder. *The Lancet*, *354*(9196), 2132–2133. https://doi.org/10.1016/S0140-6736(99)04030-1

Dreiem, A., Shan, M., Okoniewski, R. J., Sanchez-Morrissey, S., & Seegal, R. F. (2009). Methylmercury inhibits dopaminergic function in rat pup synaptosomes in an age-dependent manner. *Neurotoxicology and Teratology*, *31*(5), 312–317. https://doi.org/10.1016/j.ntt.2009.05.001

Duda, W., Wesierska, M., Ostaszewski, P., Vales, K., Nekovarova, T., & Stuchlik, A. (2016). MK-801 and memantine act differently on short-term memory tested with different time-intervals in the Morris water maze test. *Behavioural Brain Research*, *311*, 15–23. https://doi.org/10.1016/j.bbr.2016.05.024

Faro, L. R. F., do Nascimento, J. L. M., Alfonso, M., & Durán, R. (1998). Acute administration of methylmercury changes in vivo dopamine release from rat striatum. *Bulletin of Environmental Contamination and Toxicology*, *60*(4), 632–638. https://doi.org/10.1007/s001289900673

Faro, L. R. F., do Nascimento, J. L. M., Alfonso, M., & Durán, R. (2002a). Mechanism of action of methylmercury on in vivo striatal dopamine release: Possible involvement of dopamine transporter. *Neurochemistry International*, *40*(5), 455–465. https://doi.org/10.1016/S0197-0186(01)00098-5

Faro, L. R. F., do Nascimento, J. L. M., Alfonso, M., & Durán, R. (2002b). Protection of methylmercury effects on the in vivo dopamine release by NMDA receptor antagonists and nitric oxide synthase inhibitors. *Neuropharmacology*, *42*(5), 612–618. https://doi.org/10.1016/S0028-3908(02)00009-6

Faro, L. R. F., do Nascimento, J. L. M., San José, J. M., Alfonso, M., & Durán, R. (2000). Intrastriatal administration of methylmercury increases in vivo dopamine release. *Neurochemical Research*, *25*(2), 225–229. https://doi.org/10.1023/A:1007571403413

Feng, L., Li, P., & Feng, X. (2021). Methylmercury bioaccumulation in rice and health effects: A systematic review. In *Current Opinion in Environmental Science and Health* (Vol. 23). Elsevier B.V. https://doi.org/10.1016/j.coesh.2021.100285

Feng, X., Li, P., Qiu, G., Wang, S., Li, G., Shang, L., Meng, B., Jiang, H., Bai, W., Li, Z., & Fu, X. (2008). Human exposure to methylmercury through rice intake in mercury mining areas, Guizhou province, China. *Environmental Science and Technology*, *42*(1), 326–332. https://doi.org/10.1021/es071948x

Fortenbaugh, F. C., de Gutis, J., & Esterman, M. (2017). Recent theoretical, neural, and clinical advances in sustained attention research. In *Annals of the New York Academy of Sciences* (Vol. 1396, Issue 1, pp. 70–91). Blackwell Publishing Inc. https://doi.org/10.1111/nyas.13318

Gilbert, S. G., Burbacher, T. M., & Rice, D. C. (1993). Effects of in utero methylmercury exposure on spatial delayed alternation task in monkeys. *Toxicology and Applied Pharmacology*, *123*, 130–136. https://doi.org/https://doi.org/10.1006/taap.1993.1229

Goulet, S., Doré, F. Y., & Mirault, M. E. (2003). Neurobehavioral changes in mice chronically exposed to methylmercury during fetal and early postnatal development. *Neurotoxicology and Teratology*, *25*, 335–347. https://doi.org/10.1016/S0892-0362(03)00007-2

Grandjean, P., Weihe, P., Debes, F., Choi, A. L., & Budtz-Jørgensen, E. (2014). Neurotoxicity from prenatal and postnatal exposure to methylmercury. *Neurotoxicology and Teratology*, *43*, 39–44. https://doi.org/10.1016/j.ntt.2014.03.004

Grandjean, P., Weihe, P., White, R. F., Debes, F., Araki, S., Yokoyama, K., Murata, K., SØrensen, N., Dahl, R., & JØrgensen, P. J. (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicology and Teratology*, *19*(6), 417–428. https://doi.org/10.1016/S0892-0362(97)00097-4

Groth, E. (2010). Ranking the contributions of commercial fish and shellfish varieties to mercury exposure in the United States: Implications for risk communication. *Environmental Research*, *110*(3), 226–236. https://doi.org/10.1016/j.envres.2009.12.006

Izquierdo, A., Brigman, J. L., Radke, A. K., Rudebeck, P. H., & Holmes, A. (2017). The neural basis of reversal learning: An updated perspective. *Neuroscience*, *345*, 12–26. https://doi.org/10.1016/j.neuroscience.2016.03.021

Jablonski, S. A., Graham, D. L., Vorhees, C. V., & Williams, M. T. (2017). Effects of Neonatal Methamphetamine and Stress on Brain Monoamines and Corticosterone in Preweanling Rats. *Neurotoxicity Research*, *31*(2), 269–282. https://doi.org/10.1007/s12640-016-9680-y

Kalisch, B. E., & Racz, W. J. (1996). The effects of methylmercury on endogenous dopamine efflux from mouse striatal slices. *Toxicology Letters*, *89*(1), 43–49. https://doi.org/10.1016/S0378-4274(96)03787-3

Kane, M. J., & Engle, R. W. (2002). The role of prefrontal cortex in working-memory capacity, executive attention, and general fluid intelligence: an individual-differences perspective. *Psychonomic Bulletin & Review*, *9*(4), 637–671. https://doi.org/10.3758/BF03196323

Kehagia, A. A., Murray, G. K., & Robbins, T. W. (2010). Learning and cognitive flexibility: Frontostriatal function and monoaminergic modulation. *Current Opinion in Neurobiology*, *20*(2), 199–204. https://doi.org/10.1016/j.conb.2010.01.007

Kendricks, D. R., Boomhower, S. R., Arnold, M. A., Glenn, D. J., & Newland, M. C. (2020). Adolescent methylmercury exposure alters short-term remembering, but not sustained attention, in male Long-Evans rats. *NeuroToxicology*, *78*, 186–194. https://doi.org/10.1016/j/neuro.2020.03.009

Kendricks, D. R., Boomhower, S. R., & Newland, M. C. (2020). Methylmercury, attention, and memory: Baseline-dependent effects of adult d-amphetamine and marginal effects of adolescent methylmercury. *NeuroToxicology*, *80*, 130–139. https://doi.org/10.1016/j.neuro.2020.07.009

Kendricks, D. R., & Newland, M. C. (2021). Selective dopaminergic effects on attention and memory in male mice exposed to Methylmercury during adolescence. *Neurotoxicology and Teratology, 87*, 107016. https://doi.org/10.1016/j.ntt.2021.107016

Kendricks, D. R., Boomhower, S. R., & Newland, M. C. (under review). Adolescence as a sensitive period for neurotoxicity: Lifespan developmental effects of methylmercury. *Pharmacology, Biochemistry, and Behavior*.

Komulainen, H., & Tuomisto, J. (1981). Interference of Methyl Mercury with Monoamine Uptake and Release in Rat Brain Synaptosomes. *Acta Pharmacologica et Toxicologica*, *48*(3), 214–222. https://doi.org/10.1111/j.1600-0773.1981.tb01611.x

Krishna Chandran, A. M., Christina, H., Das, S., Mumbrekar, K. D., & Satish Rao, B. S. (2019). Neuroprotective role of naringenin against methylmercury induced cognitive impairment and mitochondrial damage in a mouse model. *Environmental Toxicology and Pharmacology*, *71*(April), 103224. https://doi.org/10.1016/j.etap.2019.103224

Kucinski, A., Phillips, K. B., Cherian, A. K., & Sarter, M. (2020). Rescuing the attentional performance of rats with cholinergic losses by the M1 positive allosteric modulator TAK-071. *Psychopharmacology*, *237*(1), 137–153. https://doi.org/10.1007/s00213-019-05354-5

Larsen, B., Olafsson, V., Calabro, F., Laymon, C., Tervo-Clemmens, B., Campbell, E., Minhas, D., Montez, D., Price, J., & Luna, B. (2020). Maturation of the human striatal dopamine system revealed by PET and quantitative MRI. *Nature Communications*, *11*(1), 1–10. https://doi.org/10.1038/s41467-020-14693-3

Li, P., Feng, X., & Qiu, G. (2010). Methylmercury exposure and health effects from rice and fish consumption: A review. *International Journal of Environmental Research and Public Health*, *7*(6), 2666–2691. https://doi.org/10.3390/ijerph7062666

Maximino, C., Araujo, J., Leão, L. K. R., Grisolia, A. B. A., Oliveira, K. R. M., Lima, M. G., Batista, E. de J. O., Crespo-López, M. E., Gouveia, A., & Herculano, A. M. (2011). Possible role of serotoninergic system in the neurobehavioral impairment induced by acute methylmercury exposure in zebrafish (Danio rerio). *Neurotoxicology and Teratology*, *33*(6), 727–734. https://doi.org/10.1016/j.ntt.2011.08.006

McNab, F., & Klingberg, T. (2008). Prefrontal cortex and basal ganglia control access to working memory. *Nature Neuroscience*, *11*(1), 103–107. https://doi.org/10.1038/nn2024

Mohler, E. G., Heck, W. H., & Williams, C. L. (2001). Sustained attention in adult mice is modulated by prenatal choline availability. *International Journal of Comparative Psychology*, *14*(3), 136–150. http://escholarship.org/uc/item/2d4086s1

Montgomery, K. S., Mackey, J., Thuett, K., Ginestra, S., Bizon, J. L., & Abbott, L. C. (2008). Chronic, low-dose prenatal exposure to methylmercury impairs motor and mnemonic function in adult C57/B6 mice. *Behavioural Brain Research*, *191*(1), 55–61. https://doi.org/10.1016/j.bbr.2008.03.008

Moran, P. M. (1993). Differential Effects of Scopolamine and Mecamylamine on Working and Reference Memory in the Rat. In *Pharmacology Biochemistry and Behavior* (Vol. 45).

Newland, M. C., Hoffman, D. J., Heath, J. C., & Donlin, W. D. (2013). Response inhibition is impaired by developmental methylmercury exposure: Acquisition of low-rate lever-pressing. *Behavioural Brain Research*, *253*, 196–205. https://doi.org/10.1016/j.bbr.2013.05.038

Newland, M. C., Paletz, E. M., & Reed, M. N. (2008a). Methylmercury and nutrition: Adult effects of fetal exposure in experimental models. *NeuroToxicology*, *29*(5), 783–801. https://doi.org/10.1016/j.neuro.2008.06.007

Newland, M. C., Paletz, E. M., & Reed, M. N. (2008b). Methylmercury and nutrition: Adult effects of fetal exposure in experimental models. *NeuroToxicology*, *29*(5), 783–801. https://doi.org/10.1016/j.neuro.2008.06.007

Newland, M. C., & Reile, P. A. (1999). Blood and brain mercury levels after chronic gestational exposure to methylmercury in rats. *Toxicological Sciences*, *50*(1), 106–116. https://doi.org/10.1093/toxsci/50.1.106

Newland, M. C., Reile, P. A., & Langston, J. L. (2004). Gestational exposure to methylmercury retards choice in transition in aging rats. *Neurotoxicology and Teratology*, *26*(2), 179–194. https://doi.org/10.1016/j.ntt.2003.12.004

Newland, M. C., Yezhou, S., Lögdberg, B., & Berlin, M. (1994). Prolonged behavioral effects of in utero exposure to lead or methyl mercury: Reduced sensitivity to changes in reinforcement contingencies during behavioral transitions and in steady state. *Toxicology and Applied Pharmacology*, *126*, 6–15. https://doi.org/10.1006/taap.1994.1084

Oliveira, A. N., Pinheiro, A. M., Belém-Filho, I. J. A., Fernandes, L. M. P., Cartágenes, S. C., Ribera, P. C., Fontes-Júnior, E. A., Crespo-Lopez, M. E., Monteiro, M. C., Lima, M. O., & Maia, C. S. F. (2018). Unravelling motor behaviour hallmarks in intoxicated adolescents: methylmercury subtoxic-dose exposure and binge ethanol intake paradigm in rats. *Environmental Science and Pollution Research*, *25*(22), 21937–21948. https://doi.org/10.1007/s11356-018-2235-4

Onishchenko, N., Tamm, C., Vahter, M., Hökfelt, T., Johnson, J. A., Johnson, D. A., & Ceccatelli, S. (2007). Developmental exposure to methylmercury alters learning and induces depression-like behavior in male mice. *Toxicological Sciences*, *97*(2), 428–437. https://doi.org/10.1093/toxsci/kfl199

Overman, W. H. (2004). Sex differences in early childhood, adolescence, and adulthood on cognitive tasks that rely on orbital prefrontal cortex. *Brain and Cognition*, *55*(1), 134–147. https://doi.org/10.1016/S0278-2626(03)00279-3

Paletz, E. M., Craig-Schmidt, M. C., & Newland, M. C. (2006). Gestational exposure to methylmercury and n-3 fatty acids: Effects on high- and low-rate operant behavior in adulthood. *Neurotoxicology and Teratology*, *28*(1), 59–73. https://doi.org/10.1016/j.ntt.2005.11.003

Paletz, E. M., Day, J. J., Craig-Schmidt, M. C., & Newland, M. C. (2007). Spatial and visual discrimination reversals in adult and geriatric rats exposed during gestation to methylmercury and n - 3 polyunsaturated fatty acids. *NeuroToxicology*, *28*(4), 707–719. https://doi.org/10.1016/j.neuro.2007.05.001

Pardo, J. v, Fox, P. T., & Raichle, M. E. (1991). Localization of a human system for sustained attention by positron emission tomography. *Nature*, *349*(6304), 61–64. https://doi.org/10.1038/349061a0

Peng, X., Liu, F., & Wang, W. X. (2016). Organ-specific accumulation, transportation, and elimination of methylmercury and inorganic mercury in a low Hg accumulating fish. *Environmental Toxicology and Chemistry*, *35*(8), 2074–2083. https://doi.org/10.1002/etc.3363

Rand, M. D., Conrad, K., Marvin, E., Harvey, K., Henderson, D., Tawil, R., Sobolewski, M., & Cory-Slechta, D. A. (2020). Developmental exposure to methylmercury and resultant muscle mercury accumulation and adult motor deficits in mice. *NeuroToxicology*, *81*, 1–10. https://doi.org/10.1016/j.neuro.2020.07.007

Rasmussen, E. B., & Newland, M. C. (2001). Developmental exposure to methylmercury alters behavioral sensitivity to d-amphetamine and pentobarbital in adult rats. *Neurotoxicology and Teratology*, *23*(1), 45–55. https://doi.org/10.1016/S0892-0362(00)00112-4

Reed, M. N., & Newland, M. C. (2009). Gestational methylmercury exposure selectively increases the sensitivity of operant behavior to cocaine. *Behavioral Neuroscience*, *123*(2), 408–417.

Reed, M. N., Paletz, E. M., & Newland, M. C. (2006). Gestational exposure to methylmercury and selenium: Effects on a spatial discrimination reversal in adulthood. *NeuroToxicology*, *27*, 721–732. https://doi.org/10.1016/j.neuro.2006.03.022

Roos, D. H., Puntel, R. L., Lugokenski, T. H., Ineu, R. P., Bohrer, D., Burger, M. E., Franco, J. L., Farina, M., Aschner, M., Rocha, J. B. T., & de Vargas Barbosa, N. B. (2010). Complex methylmercury-cysteine alters mercury accumulation in different tissues of mice. *Basic and Clinical Pharmacology and Toxicology*, *107*(4), 789–792. https://doi.org/10.1111/j.1742-7843.2010.00577.x

Sagiv, S. K., Thurston, S. W., Bellinger, D. C., Amarasiriwardena, C., & Korrick, S. A. (2012). Prenatal exposure to mecury and fish consumption during pregnancy and attention-deficit/hyperactivity disorder-related behavior in children. *Archives of Pediatric Adolescent Medicine*, *166*(12), 1123–1131. https://doi.org/10.1001/archpediatrics.2012.1286

Sagvolden, T., & Xu, T. (2008). l-Amphetamine improves poor sustained attention while d-amphetamine reduces overactivity and impulsiveness as well as improves sustained attention in an animal model of attention-deficit/hyperactivity disorder (ADHD). *Behavioral and Brain Functions : BBF*, *4*, 3. https://doi.org/10.1186/1744-9081-4-3

Sakamoto, M., Kakita, A., Bezerra De Oliveira, R., Sheng Pan, H., & Takahashi, H. (2004). Dose-dependent effects of methylmercury administered during neonatal brain spurt in rats. *Developmental Brain Research*, *152*(2), 171–176. https://doi.org/10.1016/j.devbrainres.2004.06.016

Sakamoto, M., Kakita, A., Wakabayashi, K., Takahashi, H., Nakano, A., & Akagi, H. (2002). Evaluation of changes in methylmercury accumulation in the developing rat brain and its effects: A study with consecutive and moderate dose exposure throughout gestation and lactation periods. *Brain Research*, *949*(1–2), 51–59. https://doi.org/10.1016/S0006-8993(02)02964-5

Sakamoto, M., Tatsuta, N., Chan, H. M., Domingo, J. L., Murata, K., & Nakai, K. (2018). Brain methylmercury uptake in fetal, neonate, weanling, and adult rats. *Environmental Research*, *167*, 15–20. https://doi.org/10.1016/j.envres.2018.06.038

Santana, L. N. da S., Bittencourt, L. O., Nascimento, P. C., Fernandes, R. M., Teixeira, F. B., Fernandes, L. M. P., Freitas Silva, M. C., Nogueira, L. S., Amado, L. L., Crespo-Lopez, M. E., Maia, C. do S. F., & Lima, R. R. (2019). Low doses of methylmercury exposure during adulthood in rats display oxidative stress, neurodegeneration in the motor cortex and lead to impairment of motor skills. *Journal of Trace Elements in Medicine and Biology*, *51*(August 2018), 19–27. https://doi.org/10.1016/j.jtemb.2018.09.004

Santos-Lima, C. dos, Mourão, D. de S., Carvalho, C. F. de, Souza-Marques, B., Vega, C. M., Gonçalves, R. A., Argollo, N., Menezes-Filho, J. A., Abreu, N., & Hacon, S. de S. (2020). Neuropsychological Effects of Mercury Exposure in Children and Adolescents of the Amazon Region, Brazil. *NeuroToxicology*, *79*, 48–57. https://doi.org/10.1016/j.neuro.2020.04.004

Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., & Nobel-Haeusslein, L. J. (2013). Brain development in rodent and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Progress in Neurobiology*, *106–107*, 1–16. https://doi.org/10.1016/j.pneurobio.2013.04.001

Spiller, H. A. (2018). Rethinking mercury: the role of selenium in the pathophysiology of mercury toxicity. *Clinical Toxicology*, *56*(5), 313–326. https://doi.org/10.1080/15563650.2017.1400555

Strain, J. J., Davidson, P. W., Bonham, M. P., Duffy, E. M., Stokes-Riner, A., Thurston, S. W., Wallace, J. M. W., Robson, P. J., Shamlaye, C. F., Georger, L. A., Sloane-Reeves, J., Cernichiari, E., Canfield, R. L., Cox, C., Huang, L. S., Janciuras, J., Myers, G. J., & Clarkson, T. W. (2008). Associations of maternal long-chain polyunsaturated fatty acids, methyl mercury, and infant development in the Seychelles Child Development Nutrition Study. *NeuroToxicology*, *29*(5), 776–782. https://doi.org/10.1016/j.neuro.2008.06.002

Tarazi, F. I., & Baldessarini, R. J. (2000). Comparative postnatal development of dopamine D1, D2, and D4 receptors in rat forebrain. *International Journal of Developmental Neuroscience*, *18*(1), 29–37. https://doi.org/10.1016/S0736-5748(99)00108-2

Tarazi, F. I., Tomasini, E. C., & Baldessarini, R. J. (1998). Postnatal development of dopamine and serotonin transporters in rat caudate-putamen and nucleus accumbens septi. *Neuroscience Letters*, *254*(1), 21–24. https://doi.org/10.1016/S0304-3940(98)00644-2

Tian, J., Luo, Y., Chen, W., Yang, S., Wang, H., Cui, J., Lu, Z., Lin, Y., & Bi, Y. (2016). MeHg Suppressed Neuronal Potency of Hippocampal NSCs Contributing to the Puberal Spatial Memory Deficits. *Biological Trace Element Research*, *172*(2), 424–436. https://doi.org/10.1007/s12011-015-0609-8

van Wijngaarden, E., Thurston, S. W., Myers, G. J., Harrington, D., Cory-Slechta, D. A., Strain, J. J., Watson, G. E., Zareba, G., Love, T., Henderson, J., Shamlaye, C. F., & Davidson, P. W. (2017). Methyl mercury exposure and neurodevelopmental outcomes in the Seychelles Child Development Study Main cohort at age 22 and 24 years. *Neurotoxicology and Teratology*, *59*, 35–42. https://doi.org/10.1016/j.ntt.2016.10.011

von Allmen, D. Y., Wurmitzer, K., Martin, E., & Klaver, P. (2013). Neural activity in the hippocampus predicts individual visual short-term memory capacity. *Hippocampus*, *23*(7), 606–615. https://doi.org/10.1002/hipo.22121

Widholm, J. J., Villareal, S., Seegal, R. F., & Schantz, S. L. (2004). Spatial alternation deficits following developmental exposure to Aroclor 1254 and/or methylmercury in rats. *Toxicological Sciences*, *82*(2), 577–589. https://doi.org/10.1093/toxsci/kfh290

Zhao, H., Yan, H., Zhang, L., Sun, G., Li, P., & Feng, X. (2019). Mercury contents in rice and potential health risks across China. *Environment International*, *126*, 406–412. https://doi.org/10.1016/j.envint.2019.02.055

Chapter 2

Neonatal Methylmercury Effects on Perseveration, Attention, Memory, and Dopamine Neurotransmission

Introduction

Early developmental exposure to methylmercury (MeHg) is linked to disruption in behavior and neurochemistry. Animal models of prenatal MeHg exposure show alterations in many behaviors, including motor function, response inhibition, perseveration, choice, and learning (Giménez-Llort et al., 2001; Newland et al., 2004; Newland et al., 2013; Onishchenko et al., 2007; Reed et al., 2006). Exposure to MeHg throughout gestation produces sex-dependent reduction in activity of monoamine oxidase (MAO), the enzyme that breaks down dopamine into the metabolite DOPAC, in rats (Beyrouty et al., 2006; Castoldi et al., 2006), and sensitivity to dopamine agonists but not dopamine antagonists or other monoamine agonists (Rasmussen & Newland, 2001; Reed & Newland, 2009). When administered acutely *in vitro*, MeHg produces inhibition of dopamine reuptake via the dopamine transporter (DAT) as well as stimulation of dopamine release from presynaptic neurons (Dreiem et al., 2009; Faro et al., 2002; Komulainen & Tuomisto, 1981).

Prenatal exposure to MeHg increases sensitivity to GABA agonists in animal models (Rasmussen & Newland, 2001). Following adolescent exposure, MeHg accumulates in the cerebellum and motor areas and produces alterations in motor coordination in rodents (Belém-Filho et al., 2018; Oliveira et al., 2018). Concurrent exposure to the GABA agonist, ethanol, reduces accumulation of MeHg in these areas (Belém-Filho et al., 2018; Oliveira et al., 2018). Postnatal exposure to moderate or very high doses of MeHg cause degradation of neurons in the cerebellum (Sakamoto et al., 2004) and hippocampus (Tian et al., 2016), areas rich in GABA and glutamate, respectively. While many of these neurotransmitter systems are affected, there are

some systems that are relatively unaffected such as norepinephrine with reports of both prenatal (Rasmussen & Newland, 2001; Reed & Newland, 2009) and adolescent (Boomhower & Newland, 2019b; Kendricks & Newland, 2021) exposure to MeHg in rodents observing no alterations in behavior in the presence of norepinephrine agonists.

MeHg impacts a variety of neurotransmitter systems and with it produces long-term alterations in behaviors mediated by these systems. Prenatal exposure to MeHg impairs animal's ability to track changes in the source of reinforcement in monkeys (Newland et al., 1994) and rats (Newland et al., 2004). MeHg's inhibition of choice may be related to other aspects of behavior, namely perseveration. Both prenatal MeHg exposure in rats (Paletz et al., 2007; Reed et al., 2006) and adolescent exposure in mice (Boomhower & Newland, 2017) produce higher rates of perseverative errors in spatial reversal tasks. Perseveration is partially mediated by activity of dopamine, serotonin, and norepinephrine with accurate prediction of reward after a reversal being sensitive to bromocriptine, a dopamine D_2 agonist (Cools et al., 2009), perseverative behavior increasing following reduction in serotonin in the orbitofrontal cortex (Clarke et al., 2004), and perseveration being partially mediated by activity within the striatum and frontal cortex (Kehagia et al., 2010; Overman, 2004), regions rich in both dopamine and norepinephrine.

While perseveration is consistently impacted by MeHg in animal models, other executive functions, such as memory and attention, are not. Memory is a complex process mediated by an array of neurotransmitters systems, not excluding glutamate, serotonin, norepinephrine, acetylcholine, and dopamine (Barros et al., 2005; Burgos et al., 2005; Bushnell & Levin, 1993; Duda et al., 2016). Impacts of MeHg on memory are variable and unconvincing in both human and animal models. Seven-year-old children living in the Faroe Island who were exposed to

MeHg throughout gestation showed deficits in verbal memory (Grandjean et al., 1997, 1998). In contrast, nine-year-old children living in the Republic of Seychelles who were also exposed during gestation showed no impairment in memory (Myers et al., 2003). This variability is not only associated with prenatal exposure. Short-term memory in 5- and 12-year-old girls of the Upper Maroni communities of French Guiana was negatively associated with MeHg concentration in the root of the hair, a measure of fairly recent exposure (Cordier et al., 2002). This was similarly reported for visuospatial memory in seven-year-old children living in the Faroe Islands (Grandjean et al., 2014). In contrast, recent exposure in adolescents and young adults in the Republic of Seychelles (Davidson et al., 2011; van Wijngaarden et al., 2017) and in 14-year-olds living in the Faroe Islands (Debes et al., 2006) was not associated with alteration in memory.

Such variability is seen also with sustained attention. Attention is largely mediated by acetylcholine and norepinephrine (Bushnell et al., 1997; Cherian et al., 2019; Kucinski et al., 2020; Mohler et al., 2001; Rezvani et al., 2002) and is partially mediated by dopamine (Cheon et al., 2003; Sagvolden & Xu, 2008). Impacts of MeHg on sustained attention vary depending on when exposure occurred. For example, prenatal MeHg exposure is associated with impaired attention in children living in the Faroe Islands (Grandjean et al., 1997) and with some symptoms of attention-deficit/hyperactivity disorder (ADHD), namely inattention, in children living in Arctic Québec (Boucher et al., 2012). However, both human and rodent models of postnatal MeHg exposure have not shown such a clear interaction (Boucher et al., 2012; Kendricks, Boomhower, Arnold, et al., 2020). While contemporaneous MeHg intoxication in children and adolescence living in Hong Kong was shown to be associated with higher prevalence of ADHD diagnosis (Cheuk & Wong, 2006), this was not true for 5-12-year-olds living near a refinery in

Omaha, NE (Kim et al., 2013), for 6-10-year-olds living in various cities in South Korea (Ha et al., 2009), or for 8-14-year-olds living in Arctic Québec (Boucher, Jacobson, et al., 2012). The lack of impact of postnatal MeHg on attention is similarly reported in animal models with rodents exposed during adolescence showing no impairment in sustained attention (Kendricks, Boomhower, & Newland, 2020; Kendricks, Boomhower, Arnold, et al., 2020). However, it is unclear at this point whether prenatal exposure to MeHg in rodents would interact with attentional processes.

MeHg's impacts on perseveration are well established following pre-natal exposure. This is not the case for attention and memory. It could be that the variability in effects of MeHg on attention and memory is due to differences in nutrients obtained from fish consumption (Burger et al., 2012; Strain et al., 2008) as the source of fish varies among the Seychellois, Maronis, and Faroese communities (Davidson et al., 1998; Fréry et al., 2001; Grandjean et al., 1992). Another possible explanation is the age at which behavioral markers were observed, *i.e.*, childhood versus adolescence/young adulthood, with childhood and early adolescence being more sensitive to MeHg's neurotoxic effects than late adolescence and early adulthood. This would suggest that the age at which exposure occurs is essential to the determination of relative sensitivity to MeHg toxicity.

MeHg's toxicity depends on the age at which exposure occurred, with some developmental periods, such as gestation, being markedly more sensitive than other periods. However, one period of development has been consistently overlooked. In rodents, neural changes occurring during early postnatal development, the neonatal period, are akin to those changes occurring during the third trimester of pregnancy in humans (Semple et al., 2013). This period in rodents is often modeled by continuing maternal MeHg exposure, presumably allowing

pups to be exposed to MeHg via the mother's milk during lactation. Milk, however, is not a substantive source of MeHg exposure as mercury in breast milk is not bioavailable (Newland & Reile, 1999; Newland et al., 2008; Sakamoto et al., 2002). While some reports do expose neonates to MeHg, these reports are often confounded by exposure continuing into adolescence (Tian et al., 2016) or do not directly model human exposures due to the high doses employed (Sakamoto et al., 2004). Because developmental periods are differentially sensitive to MeHg's effects, the absence of reports of behavior that isolate this key window of development in rodents is a significant drawback in drawing comparisons between rodent and human models of prenatal MeHg's toxicity.

In order to determine if the neonatal period is similarly sensitive to MeHg toxicity as the prenatal or adolescent periods, behavioral and neurochemical changes were evaluated in male rats exposed to 0, 30, or 130 ppm Hg from postnatal day (PND) 1 to 10, the span of the neonatal period in rodents. These doses were selected to closely approximate those previously used in our lab to examine MeHg's developmental neurotoxicity following exposure during gestation and adolescence. Perseveration, short-term memory, and sustained attention were assessed in these rats during adulthood. MeHg exposure has been linked to alterations in reuptake of dopamine, suggesting an impact of MeHg on the dopamine transporter, DAT, (Dreiem et al., 2009; Faro et al., 2002). Therefore, DAT expression in brain regions associated with these behaviors was also assessed, namely in the prefrontal cortex, striatum, and hippocampus.

Methods

Subjects

Subjects were 74 male Long Evans rats exposed to MeHg from PND 1-10. Subjects were from two separate cohorts: One designated for analyses of markers of dopamine function on

PND 91 (Fig. 1A) and another for behavioral assessment beginning on PND 91 (Fig. 1C). Subjects were offspring of untimed pregnant dams purchased from ENVIGO (Indianapolis, IN). Dams had *ad libitum* access to standard animal chow and water and were singly housed in standard shoebox cages in an AAALAC-approved animal facility with controlled temperature/humidity and a 12:12 light/dark cycle (lights on at 6:00am). Upon arrival, dams were weighed and each morning dams were checked to determine if a litter was born. The day of birth for pups (designated as after 5:00 pm of the preceding day) was PND 0. Upon birth, pups were sexed, weighed, and three males were marked for exposure. On PND 2, litters were culled to 8 pups, ensuring equal numbers of each sex when possible. Pups were cross-fostered from larger litters when a litter did not have enough male pups for exposure. Pups were weaned on PND 21 and were pair-housed in ventilated rat cages (Thoren, Maxi-Miser Caging System; Hazelton, PA). After weaning, pups were provided *ad libitum* access to tap water throughout life and *ad libitum* access to standard animal chow until approximately PND 75 or when rats exceeded 300g body weight, when they were placed under caloric restriction to maintain a stable body mass of 300g. Body mass was maintained by feeding rats approximately 12(±2)g food/rat/day.

Exposure

Neonatal rats were exposed to approximately 0, 30, or 130 ppm methylmercuric chloride (MeHgCl) dissolved in a 9:1 water to PediaSure® solution from PND 1-10 (Figure 1A; 1C). The MeHg solution was mixed by first dissolving MeHgCl in tap water and then diluting 9:1 in PediaSure®. The control, 0 ppm MeHg, was a 9:1 water to PediaSure® solution. During exposure, pups were separated from the dam 30 min prior to dosing and placed on a warm pad to prevent loss of body heat. Exposure occurred orally via pipette (VWR) in a dose of 2.5 µL/g

body weight for animals euthanized on PND 91 for dopamine analysis, and 3 µL/g body weight for animals who began behavioral testing on PND 91. After dosing, rats were returned to the warm pad for an additional 5 min before being returned to the dam. Animals were weighed daily during the exposure period to both ensure accurate dosing and to ensure the pups were gaining sufficient weight during this sensitive period despite being separated from the dam daily. Exposure ended on PND 10 with pups being regularly separated from the mother, at least three times per week for weighing, until PND 21.

Behavioral Assessment

Apparatus

Behavioral procedures were conducted in twelve Med-Associates® operant chambers (Model ENV-007, St. Albans, VT) equipped with two retractable levers located on the left and right sides of the front wall and a non-retractable lever located in the center of the rear wall. An incandescent light sat above each of the right and left front levers. In the center of the front wall, between the retractable levers, was a magazine and pellet dispenser that dispensed two 20mg sucrose pellets when response criteria were met. A central house light was located on the top front panel, above the magazine. On the left and right sides of the house light were Sonalert® generators, one with a high tone of 4500Hz and the other a low tone of 2900Hz. Each operant chamber was encased in a sound attenuating box with a fan that provided white noise throughout behavioral sessions. A computer located in a separate room controlled all experimental parameters.

Autoshaping and Chain Training

On PND 91, 36 animals (12 per exposure group; Fig. 1C) were trained to lever-press for access to sucrose reinforcement using an autoshaping procedure described previously (Pope et

al., 2016; Reed et al., 2006). Training began on either the left or right front lever, counter-balanced across animals and exposure groups. Either the left or right front lever was inserted into the operant chamber with its corresponding stimulus light illuminated. After either 30s or a response on the inserted lever, two 20mg sucrose pellets would be dispensed, followed by a 5min inter-trial interval (ITI). Ten reinforced responses on the presented lever ended free reinforcement and sucrose delivery became contingent on lever pressing under a fixed ratio 1 (FR1) reinforcement schedule. Once the rat completed 40 reinforced responses under FR1 the trained lever switched with criterion being 40 reinforced responses under FR1 on the new lever. After pressing the right and left front levers was established, pressing the central rear lever was trained, also beginning on FR1 to the same criterion of 40 reinforced responses.

Following autoshaping, animals were trained to track responding on either the left or right front lever in a chain training procedure. In chain training, each trial was signaled by a pulsating tone and a press of the rear lever within 5 minutes of trial onset caused the insertion of one of the two front levers into the chamber. A response on the available lever resulted in sucrose reinforcement and a 10s ITI before the initiation of the next trial. Failure to respond to the presented lever within 5 min was counted as an omission. Presentation of left and right levers was pseudorandom across a session, each being presented an equivalent number of times. Chain training was considered complete when 50 reinforced responses occurred in each of three consecutive sessions.

Spatial Discrimination Reversal

Behavioral flexibility was assessed in a spatial discrimination reversal (SDR) procedure (Paletz et al., 2007; Pope et al., 2016). SDR occurred in four phases: an original discrimination (OD) in which animals made a single response on either the left or right front lever

(counterbalanced across rats) resulted in sucrose reinforcement; a first reversal (R1), in which the lever that provided reinforcement was switched (*e.g..,* if OD was on the left, the lever would switch to the right during R1); a second reversal (R2), in which the lever was switched back to the OD lever; and a third reversal (R3) in which the lever switched once more to the R1 lever. A trial was signaled by a pulsating tone. A press on the rear lever within 5s of trial start silenced the tone and caused both the right and left front lever to be inserted into the chamber. A press on the designated lever (left or right), a "correct" response, resulted in reinforcement and a press on the other lever, an "incorrect" response, resulted in a 10s ITI without reinforcement. An omission was counted if there was either a failure to respond on the rear lever to initiate the trial or a failure to respond on a presented lever within 15s of the trial start. Criterion for initiating a reversal was over 85% responding (≥ 51 responses occurred) on the reinforcing lever in each of three consecutive sessions. Once the criterion was met, the lever for reinforcement switched until the criterion of at least 85% of responses occurred on the newly designated lever for three consecutive sessions. SDR was complete when criterion was met on the third reversal.

Visual Signal Detection

Following completion of SDR, animals were trained to track and discriminate between the presence and absence of a 0.3s signal light using a fading procedure (Kendricks, Boomhower, Arnold, et al., 2020). In signal detection training, a trial was initiated by a 1s low tone followed by presentation of either the left or right signal light occurring 0.3s prior to insertion of the left and right front levers. During the initial training trial, the signal light remained illuminated for 30s after lever insertion or until a response occurred. The signal light, and corresponding response, were counterbalanced across animals and groups. For animals whose signal light was the left light, responding on the left lever when the signal was presented

and the right lever when the signal was not presented resulted in sucrose reinforcement. The reverse occurred for animals whose signal was the right light. With each correct response, the duration of the signal light decreased by 1% until the signal was illuminated for 0.3s prior to lever insertion and for less than 0.1s after lever insertion. Once this criterion was met, the signal's duration was set to 0.3s (prior to the levers) and the rat was required to complete three consecutive sessions with at least 85% accuracy. Incorrect responses and omissions resulted in a 3s time-out (TO), return to a 10s ITI, and subsequently a correction procedure where the same trial type was presented repeatedly until a correct response was made.

Training continued under a percentile schedule for response latency (Kendricks, Boomhower, Arnold, et al., 2020): reinforcement was made available only when the latency to respond, the time between the levers being inserted and a response occurring, was less than 25% of the previous 10 latencies. This maintained a consistent 75% reinforcement rate, constantly encouraged shortening response latency, reduced variability in choice latencies, and continued reinforcement even if performance deteriorated.

Sustained Attention/Remembering

Following completion of signal detection training, rats were required to attend to the 0.3s signal and to remember whether the signal had occurred or not (Fig. 2 Left). A trial commenced with a 1s low tone followed by an attending delay that was randomly selected, without replacement, from a pool of 12 delays. The attending delay ensured that timing of the signal was unpredictable. Two sets of attending delays were used and they alternated between sessions. Delays in set 1 included: 0.5, 2.2, 3.7, 10, 27.2, and 44.9s. Delays in set 2 included: 0.3, 0.8, 1.4, 6.1, 16.5, and 74s. Following the attending delay, either the 0.3s signal occurred (signal trial) or it did not occur (blank trial). Another delay followed, with that delay being either 2, 3, or 4s.

Animals completed 14 sessions with these delays with the final four sessions used to assess animal's attention to the visual signal.

Following 14 sessions with 2-4s delays occurring after the signal, longer delays were introduced to assess memory of whether the signal occurred or did not occur (Fig. 2 Left). Here, a trial commenced with a 1s low tone followed by an attending delay randomly selected without replacement from one of the two delay sets. After the attending delay elapsed, either the signal was presented (signal) or it was not presented (blank) followed by a retention delay randomly selected without replacement from a pool of 6 delays. Retention delays ranged between 0.3 and 29.3s and were divided into two sets of three delays each. Delays in the first set were: 0.3, 1.9, and 11.7s. Delays in the second set were: 0.8, 4.7, and 29.3s. Animals completed an additional 12 sessions with these delay sets with the final four sessions used to determine animals' retention of the visual signal.

Latencies during attention/remembering were maintained under a percentile schedule, both to minimize variability in choice latency as well as to minimize the effect of choice latency on altering accuracy following the retention delay.

Immunoblotting

Concentration of the dopamine transporter (DAT) was assessed in the striatum and prefrontal cortex (PFC) of 91-day-old rats (N = 19; Fig. 1A). DAT was also assessed in the PFC, striatum, and hippocampus of 18 rats, approximately 8 months of age, who previously underwent behavioral testing (Fig. 1C). Animals were euthanized with CO_2 and brains were immediately removed, washed in ice cold PBS buffer, and the prefrontal cortex, striatum, hippocampus, and cerebellum were dissected on an ice-cold plate. Samples were digested in RIPA buffer (Sigma #R0278) with 1% protease inhibitor cocktail (Sigma #P8340). Protein concentration in tissue

was assessed using bicinchoninic acid assay (VWR #PI23227) and 20ug of protein was loaded and run on 4-15% Mini-Protean precast gels (Bio-Rad #4561083) with precision plus protein standards (Bio-Rad #1610376) as the ladder. Gels were run at a constant 100V for 85 minutes and then transferred onto 0.45µm PVDF membranes overnight at a constant 30V at 4ºC. Membranes were washed with TBST, blocked for 1hr with 5% NFDM, and incubated overnight at 4ºC with rabbit anti-DAT (1:1000, Sigma #D6944) or mouse anti-β-actin (1:8,000, Sigma #A5441) antibodies. Membranes were washed and incubated with appropriate horseradish peroxidase linked secondary antibodies (1:60,000 goat anti-rabbit IgG Cell Signaling #7074P2 or 1:80,000 rabbit anti-mouse IgG Sigma #A9044) and visualized using west pico PLUS ECL detection reagent (VWR #PI34577) and a gel imager system. Band density was assessed using NIH ImageJ software.

MeHg Analysis in Tissue and Water Solutions

Concentration of MeHg was assessed in whole brain tissue of pups at 12 or 13 days of age (N = 19; Fig. 1B), 2-3 days after the end of exposure to allow sufficient time for MeHg in the blood to wash out while maintaining adequate brain Hg concentration. Pups were euthanized with CO_2 and whole brains were dissected. Water samples were taken at the end of exposure for both Cohort 1 and Cohort 2. MeHg concentrations were assessed using inductively coupled plasma mass spectrometry (ICP-MS) at Michigan State University's Veterinary Diagnostic Laboratory.

Data Analysis

Data management and analyses were performed using the tidyverse (v1.2.1), nlme (v3.1-140), and lme4 (v1.1-21) packages running on R (v4.1.0).

Primary response variables during SDR included correct responses, incorrect responses, and omissions occurring at each reversal. Data from the last three sessions for OD, R1, and R2 were used to determine baseline responding prior to each reversal. The impact of MeHg on the transition in responding at each reversal was assessed by applying the self-starting function SSlogis which fits a three-parameter logistic function of the form (Eq. 1):

$$p(Correct) = \frac{Y_{max}}{1 + e^{\left(\frac{(X_{mid} - X)}{scale}\right)}} \quad \text{Eq. 1}$$

Where Y_{max} is the magnitude of the transition, or the maximum achieved accuracy after the transition. A higher Y_{max} denotes higher achieved accuracy on the new lever after the reversal. X_{mid} is the inflection point of the curve, or the number of trials to achieve half of the transition. A higher X_{mid} is indicative of more trials being necessary for the transition to occur. Scale describes the growth rate of the transition and is synonymous with the negative inverse of the slope, k. In other words, it is the time required to get from 1/*e* to 2/*e* trials. Since *e*, the base of the natural logarithm, is approximately 2.73, this means that it describes how much time or how many trials are required to get from about ¼ to ¾ of the way through the transition. A larger value for scale is indicative of a slower growth rate (more time required) during the middle phase of the transition. The impact of MeHg on each of these parameters with was assessed across data for R1, R2, and R3 using a non-linear mixed effect model with the random effect allowing all three parameters to vary by subject. Prior to each transition, correct responses were coded as "0" and incorrect responses were coded as "1". After the transition, correct responses were coded as 1 and incorrect responses were coded as 0. Coefficients were isolated for each of these parameters at each reversal and one-way ANOVA was used to determine differences across MeHg groups. Results were considered significant if $p < 0.05$.

Primary response variables during attention/remembering included correct responses when the signal was presented (hits), correct responses when the signal was not presented (correct rejection, CR), incorrect responses when the signal was presented (miss), and incorrect responses when the signal was not presented (false alarm, FA) (Fig. 2 Right). Total omissions and median choice latency for each session were also recorded and assessed separately. Data were Winsorized to manage outliers without removing data points. The primary dependent variables for accuracy during attention/remembering were the hit rate, p(Hit) (Eq. 2a) and the FA rate, p(FA) (Eq. 2b).

$$p(Hit) = \left(\frac{Hits}{Hits + Miss}\right) \quad \text{Eq. 2a}$$

$$p(FA) = \left(\frac{FA}{FA + CR}\right) \quad \text{Eq. 2b}$$

During acquisition of the visual signal detection procedure linear mixed effects (LME) models were used to assess the impact of MeHg on both p(Hit) and p(FA) across sessions. The change in p(Hit) across session was modeled using a quadratic equation and the change in p(FA) was measured across the inverse of session, or 1/session. During attention/remembering, both p(Hit) and p(FA) were assessed across retention delays, averaged across attending delays. LME was also used to assess the effects of MeHg on both p(Hit) and p(FA) across retention delays. A linear function was used to model p(FA) across retention delays and a quadratic function fit to p(Hit) across retention delays. Choice latency during the acquisition phase of attention was modeled using a log-linear function with choice latency assessed across the log of session. As with SDR, results were considered significant if $p < 0.05$.

DAT expression was calculated as a percent of the loading control, β-actin. In cases where outliers were detected, data were Winsorized. Differences in DAT expression in the

striatum, PFC, and hippocampus of adult rats were assessed using one-way ANOVA. Results were considered statistically significant if $p < 0.05$.

Results

Mercury Content in Water and Tissue

Animals were exposed to approximately 0, 30, or 130 ppm MeHg from PND 1-10 with whole brain concentration of MeHg assessed on PND 12 or 13 (Fig. 1B). Body mass did not differ among exposure groups from PND 1-75 for either those animals that were euthanized on PND 91 for dopamine analyses (Fig. 3A) or those animals that underwent behavioral testing beginning on PND 91 (Fig. 3B). Brain concentrations of MeHg increased dose-dependently with higher concentrations observed in those animals exposed to 130 ppm MeHg compared to 30 ppm MeHg. No detectable MeHg was observed in animals exposed to 0 ppm MeHg (Fig. 3C). Whole brain MeHg levels in those animals exposed to 130 ppm MeHg were approximately 4X the amount observed in animals exposed to 30 ppm showing that brain Hg content reliably tracked dose.

Behavioral Assessment

Effects on SDR

S-Figure 1 shows responding across all three reversals from three representative animals, one from each exposure group. A lever-press that was reinforced during a transition was coded as "1" and the other lever-press was coded as "0." Note that lever-presses coded as "0" were reinforced on previous trials (designated by negative numbers). Accuracy was coded as "0" prior to the reversal and "1" after the reversal. The panels show the transition in responding for the first, second, and third reversals. These transitions were modeled using a three-parameter logistic

equation (Eq. 1). The trial where the transition occurred is demarcated as Trial "0" on the X-axis. Dots on the top and bottom of each figure show individual responses emitted by each animal. Animals transitioned slowest on the first reversal and fastest on the third reversal in all groups.

Fig. 4A shows transitions modeled by Eq. 1 for the first, second, and third reversals across all MeHg exposure groups. Data at each reversal are averaged across animals in each group. One animal in the 0 ppm group was excluded from analyses because of equipment problems during the first reversal during testing leaving an N of 12 for the 30 and 130 ppm groups and 11 for the 0 ppm group. For Reversal 1 (Fig. 4A), there was a significant main effect of MeHg on Ymax: the animals exposed to 30 ppm MeHg ($t(16937) = -2.126$), $p = 0.034$) had a slightly smaller Ymax than the others. There was no effect of MeHg on Xmid or scale at this reversal. For Reversal 2, there was a significant main effect of MeHg both on Ymax and on scale. This effect was seen both by a smaller Ymax ($t(15737) = -2.678$, $p = 0.007$) and a smaller scale, or steeper slope ($t(15737) = -2.398$, $p = 0.017$), for animals exposed to 130 ppm MeHg. There was no effect of MeHg on Xmid. Finally, for Reversal 3, there was a significant main effect of MeHg on scale with this effect seen in an increase in scale, or a shallower slope, for animals exposed to 130 ppm MeHg ($t(15017) = 2.913$, $p = 0.004$). There were no effects of MeHg on either Ymax of Xmid at this reversal.

In order to assess MeHg-related alterations in behavioral patterns at each of these reversals better, parameter estimates were isolated for Ymax, Xmid, and scale at each reversal. Fig. 4B shows mean parameter estimates at each reversal for each of the exposure groups. For Ymax, there was a significant main effect of MeHg at all three reversals. For Reversal 1 and Reversal 3, animals exposed to 30 ppm MeHg differed from those exposed to either 0 or 130 ppm MeHg but this effect was small and in opposing directions. Animals exposed to 30 ppm had

lower achieved Ymax during Reversal 1 ($t(32) = -4.012$, $p = 0.0003$), suggesting a less robust transition to responding on the new lever. However, Ymax was higher in these animals for Reversal 3 ($t(32) = 3.566$, $p = 0.001$) suggesting an effect of MeHg on perseveration that is dependent on prior exposure to each reinforcement alternative. For Reversal 2, this effect lay with animals exposed to 130 ppm MeHg, who, overall, achieved lower Ymax than animals exposed to either 0 or 30 ppm MeHg ($t(32) = -4.285$, $p = 0.0002$) suggesting that switching back to the originally trained lever is somehow impaired by a high dose of MeHg. It is concluded here that MeHg impacts the degree to which responding reliably switches after a transition and that this effect depends on prior exposure to each reinforcement alternative.

For Xmid, there was no significant effect of MeHg at any reversal. For scale, there was a significant main effect of MeHg at Reversal 2 and Reversal 3, but not Reversal 1. Animals exposed to 130 ppm MeHg differed from those animals exposed to 0 or 30 ppm MeHg. This effect, again, was in opposite directions depending on the reversal. Animals exposed to 130 ppm MeHg had an overall smaller scale, indicative of a steeper slope, during the transition in Reversal 2 ($t(32) = -2.552$, $p = 0.016$). This suggest that exposed animals tend not to perseverate on the previously novel lever. Animals exposed to 130 ppm MeHg had an overall larger scale, or a shallower slope, during transition in Reversal 3 ($t(32) = 3.125$, $p = 0.004$). This suggests that these animals tended to perseverate on the originally trained lever, unlike the novel lever. Note that the second reversal, which the exposed animals completed quickly, was to the lever on which the position discrimination was originally trained and the third reversal, which the exposed animals completed relatively slowly, was to the other lever.

Effects on Visual Signal Detection

Figure 5 shows changes in accuracy and choice latency during the acquisition of visual signal detection. Accuracy was modeled using using p(Hit) (Eq. 2a), a measure of accurate responding in the presence of the signal, and p(FA) (Eq. 2b), a measure of accurate responding in the absence of the signal. Accuracy was measured across sessions as the duration of the visual signal decreased. As expected, p(Hit) (Fig. 5A) increased during training reaching a high rate in all animals at the end of the training period, similar to previous reports (Kendricks, Boomhower, Arnold, et al., 2020). This effect, however, was not impacted by MeHg ($F(4, 498) = 1.442$, $p = 0.219$). On the other hand, p(FA) (Fig. 5B) decreased rapidly early in training before leveling off to a stable low rate. This effect was dependent on MeHg: animals exposed to 130 ppm MeHg had a shallower downward shift in p(FA) early in training and an overall higher p(FA) at the end of training ($t(501) = -2.795$, $p = 0.005$), yielding a slower acquisition.

During visual signal detection, animals were placed on a percentile schedule for choice latency to both drive down and minimize variation in choice latency. In order to observe the efficacy of this, and to determine if there was any baseline variation in choice latency among MeHg exposure groups, choice latency was measured during this training period. An exponential model was used to model latency changes across sessions. Choice latencies reliably decreased for all animals throughout the training period (Fig. 5C). A significant interaction occurred between session and MeHg exposure. Animals exposed to both 30 ppm MeHg ($t(501) = -2.109$, $p = 0.035$) and 130 ppm MeHg ($t(501) = 3.389$, $p = 0.001$) differed from animals exposed to 0 ppm MeHg and from each other. This is seen in a shallower downward shift in latencies for animals exposed to 130 ppm MeHg and a steeper downward shift in latencies for animals exposed to 30 ppm MeHg.

Sustained Attention

S-Figure 2 shows p(Hit) and p(FA) during the first part of the sustained attention procedure, in which delays of 2, 3, or 4s occurred after the termination of the visual signal or blank pause to assess sustained attention in the absence of memory. For p(Hit) (S-Fig. 2A), there was no main effect of MeHg or of delay (2, 3, or 4s) but there was a borderline significant interaction between MeHg and delay ($F(2, 69) = 2.885$, $p = 0.062$). For p(FA) (S-Fig. 2B), there was no effect of either delay or of MeHg, regardless of delay being in the model. These data suggest that there was no impact of MeHg on sustained attention.

Both median choice latency and the total number of omissions per session, averaged across sessions, were examined during sustained attention (S-Fig. 3A). Animals exposed to 30 ppm MeHg had overall slightly more omissions than animals exposed to either 0 or 130 ppm MeHg (Fig 11A, $t(23) = 3.343$, $p = 0.003$), but this should be interpreted cautiously considering the small number of omissions emitted by these animals. There was no effect of MeHg on choice latency (S-Fig. 3B) during this phase meaning that variability in animal's latency to respond to the lever, *e.g.*, any post-signal delay introduced because an animal took a longer time to respond, did not reliably interact with their attention to the signal.

Remembering

Figure 6 shows shifts in p(Hit) and p(FA) across the retention delays (0.3-29.3s) after the long delays were introduced. As expected from earlier reports (Kendricks, Boomhower, Arnold, et al., 2020), p(Hit) (Fig. 6A) varied across retention delays with a higher p(Hit) at shorter delays and a steady decline in p(Hit) as the delay increased ($F(2, 274) = 146.140$, $p < 0.0001$). This trend is indicative of an effect of training delay on peak accuracy and an expected decline in accuracy due to forgetting of the signal's presence (Kendricks, Boomhower, Arnold, et al.,

2020). This effect, however, was not dependent on MeHg nor was there a main effect of MeHg suggesting that MeHg affected neither peak accuracy nor memory. Also as reported previously, p(FA) (Fig. 6B) did not vary across delay nor was there an effect of MeHg on p(FA).

As has been reported previously (Kendricks, Boomhower, & Newland, 2020; Kendricks, Boomhower, Arnold, et al., 2020), p(Hit) tended to peak at the 1.9s delay, the training delay, and not at the shortest delay of 0.3s. In S-Figure 4 we isolate accuracy at these two delays. As seen in S-Fig 4A, p(Hit) is higher at the 1.9s delay ($F(1, 33) = 16.476$, $p < 0.0001$), supporting previous data of an influence of training condition in determining the peak accuracy in this procedure (Kendricks, Boomhower, & Newland, 2020; Kendricks, Boomhower, Arnold, et al., 2020). There was no effect of MeHg at these delays, lending further support of a lack of effect of MeHg on attention. For p(FA) (S-Fig. 4B), there was no difference between these delays and there was no effect of MeHg.

Omissions and choice latency were also isolated for the remembering procedure. These data are shown in S-Figure 5. The number of omissions that occurred at each retention delay was assessed (S-Fig. 5A). Omissions did not significantly vary across delay but there was a trend of more omissions occurring at longer delays ($t(179) = 1.801$, $p = 0.073$). Omissions were dependent on MeHg, with animals exposed to 30 ppm MeHg having a higher number of omissions ($t(23) = 2.322$, $p = 0.029$) than animals exposed to either 0 or 130 ppm MeHg. However, again this should be interpreted cautiously given the very small number of omissions these animals committed. Overall choice latency (S-Fig. 5B) did not vary across MeHg groups.

Monoamine Analyses

In order to determine if MeHg during the neonatal period resulted in long-term changes in dopamine neurotransmission, DAT concentration was assessed in the striatum and PFC of rats

euthanized on PND 91. DAT concentrations in these rats were normalized to the loading control, β-Actin. DAT in the striatum did not vary by MeHg exposure ($F(2,16) = 0.04$, $p = 0.959$; Fig. 7A and B). There were also no MeHg-related differences in expression of DAT within the PFC ($F(2,15) = 0.74$, $p = 0.952$; Fig. 7C and D).

Concentration of DAT in various brain regions was also assessed in rats following the completion of behavioral training at approximately 8 months of age. DAT was assessed in the striatum, PFC, and hippocampus of these adult rats. DAT concentrations were normalized to the loading control, β-Actin. Similar to in animals on PND 91, DAT did not vary by MeHg exposure within the striatum ($F(2, 13) = 0.23$, $p = 0.795$; Fig. 8A) or the PFC ($F(2, 14) = 0.09$, $p = 0.913$; Fig. 8B and D). However, in the hippocampus DAT concentration tended to trend across MeHg groups with somewhat reduced expression of DAT in animals exposed to 30 ppm MeHg. This trend was noted, however it was not found be significant ($F(2,14) = 2.79$, $p = 0.095$; Fig. 9C).

Discussion

Neonatal MeHg exposure produced perseveration and impairment in acquiring the attention/remembering task. Those rats exposed to MeHg displayed altered patterns of transition during the second and third reversals of the SDR procedure. Further, those rats exposed to the highest dose of MeHg acquired the visual signal detection paradigm slower, and to a lesser accuracy, than rats exposed to no or little MeHg. These results are akin to previous reports of behavioral detriment associated with developmental MeHg exposure. This is seen in impairment in the SDR procedure which has been shown following both gestational and adolescent exposure in rodents (Boomhower & Newland, 2017; Paletz et al., 2007) as well as impairment in learning

of the visual signal detection procedure which was previously observed in rats following adolescent exposure (Kendricks, Boomhower, Arnold, et al., 2020).

Neonatal Methylmercury and Growth and Brain Hg

Rats in this study were exposed to approximately 30 or 130 ppm MeHg from PND 1-10. This resulted in a daily dose of approximately 80 or 350 µg/kg/day Hg with variation attributed to both a difference in water concentration between cohorts 1 and 2 as well as a difference in the amount of MeHg solution administered between these two cohorts. These concentrations are similar to the doses used by Paletz and colleagues (2007), Boomhower and Newland (2017), or Kendricks and colleagues (2020). Paletz et al. (2007) and Kendricks et al., (2020) used a low dose of approximately 40 µg/kg/day and all three studies used a high dose of 400 µg/kg/day. The low dose used in the current study was approximately twice that used in previous studies, but the high dose used was more similar. Resulting brain concentrations in these rats tracked these doses with animals exposed to 130 ppm MeHg having brain concentrations of four times that of those animals exposed to 30 ppm MeHg. However, the short exposure duration used in the current study likely did not result in a stabilization of brain Hg which, in pregnant rats, occurs after approximately 28 days of exposure (Newland & Reile, 1999). This short exposure duration may be the cause of the subtlety of effects observed in this study. It is possible that toxicity of MeHg is determined by not only the dose of exposure but also the time-course of exposure with longer periods of exposure to lower doses capturable by shorter exposure durations to higher concentrations (Piotrowski & Buchanan, 1982). Therefore, it may be the case that the doses used in the current study, which were akin to previous studies using a longer exposure duration, upwards of 40 days (Boomhower & Newland, 2017; Kendricks & Newland, 2021; Paletz et al.,

2007), were too low to produce a substantial effect with the exposure durations used. It might be noted that the duration used was driven by an interest in modeling the third trimester of human pregnancy. However, it may also be that the early neonatal period is relatively insensitive to MeHg toxicity when compared to other developmental windows, but that cannot be addressed adequately in this paper.

Neonatal Methylmercury on Perseverative Behavior

Exposure to the high dose of MeHg during this period produced mild, but important, alterations in behavior in adult rats. The rate of transition during the SDR procedure, often modeled by either the slope of the transition or the number of trials to meet criterion during reversals in this phase, has consistently been shown to be disrupted by MeHg when exposure occurs during other developmental periods. For example, female rats exposed to MeHg during gestation took longer to reach criterion on the first reversal of this spatial discrimination reversal procedure (Paletz et al., 2007; Reed et al., 2006) while male mice exposed to MeHg during adolescence took more trials to reach criterion on the second reversal in this task (Boomhower & Newland, 2017). These results are similar to the effects seen in animals of the current study. While the number of trials to reach criterion in these animals was not affected, the slope of the transition, marking the rate at which a transition occurs once it begins, during each reversal was.

MeHg-exposed animals had a steeper slope during the second reversal, suggesting a rapid transition in responding, and a shallower slope during the third reversal, suggesting a slower transition in responding. This trend is important as it suggests a difference in the rate of transition depending on the initial training condition with these animals more quickly transitioning when the task conditions matched the initial training (*i.e.*, R2 matches OD) but transitioning more slowly when the task conditions did not match that initial training (*i.e.*, R3

matches R1). This trend is similar to that reported by Paletz and colleagues (2007) who similarly reported an impact of MeHg in the transition to the novel lever, but not the familiar one. These findings, together, suggest that perseveration on the novel, or new, response alternative is affected by neuronal changes occurring across the late prenatal and early postnatal periods in rodents. The neuroanatomical and neurophysiological changes occurring during the rodent neonatal period closely resemble those occurring during the third trimester of human pregnancy (Semple et al., 2013) so it may be the case that disruption during the third human trimester would have similar behavioral consequences. That said, in isolation, as in the present study, the neonatal period may be less sensitive than the prenatal period, as evidenced by the relatively small changes in behavior reported here.

Neonatal Methylmercury on Visual Signal Detection

Rats exposed to MeHg during adolescence were slower to acquire accurate reporting of the signal's absence, seen in a shallower transition in false-alarm rate early in training (Kendricks, Boomhower, Arnold, et al., 2020). This trend is similarly observed here with rats exposed to the highest dose of MeHg more slowly acquiring accurate rejection of the signal when the signal was not presented. Similarly, both those rats exposed during adolescence (Kendricks, Boomhower, Arnold, et al., 2020) as well as rats of the current study exposed during the neonatal period, did not show any such impairment in the rate of acquisition of hits, or accurate reporting of the signal's presence. This suggests that MeHg selectively impairs accurate reporting when the signal is not presented, a phenomenon that is also seen after activation of glutamate NMDA receptors by acute infusions of NMDA into the basal forebrain of adult rats (Turchi & Sarter, 2001). Therefore, it may be the case that postnatal exposure to MeHg negatively impacts maturation of glutamate neurotransmission.

Animals in the current study were placed under a percentile schedule of reinforcement during acquisition and maintenance of the attention/remembering task. This training mainly served to reduce variability in response latencies, and thus variability in the delay between when the signal light was presented and when a response occurred to indicate retention of that signal's presence. Because of the way the percentile schedule works, it also served to maintain a steady reinforcement rate even when variation in latencies did occur. It was imperative that both animals' choice latencies as well as the variability of those latencies be reduced during training. As is evident in Figure 6C, choice latencies and their variability reliably decreased. Even with the tight control over behavior afforded by the percentile schedule, MeHg-related differences in the reduction in choice latency were observed. Animals exposed to a lower dose of MeHg started training off with slightly longer choice latencies but then more quickly came under the control of the percentile schedule. On the other hand, animals exposed to the higher dose of MeHg began training with slightly shorter choice latencies and then more slowly came under the control of the percentile. This may suggest a difference in motor function or reinforcement processing between these groups as choice latencies under this percentile drive the availability of reinforcement so maintaining responding under this percentile would result in reinforcement being delivered. Therefore, those animals that more quickly come under the control of the percentile may be better at tracking reinforcement availability. MeHg has been shown to impact reinforcement processing in rodents exposed to MeHg during adolescence (Boomhower & Newland, 2019a, 2019b) and it severely impacts choice in both nonhuman primates (Newland et al., 1994) and rodents (Newland et al., 2004), with choice contingent on processing reinforcement availability. Therefore, it may be the case that changes in reinforcement processing arise following exposure to MeHg across early development.

Neonatal Methylmercury on Sustained Attention and Short-Term Memory

While impairment in sustained attention is associated with prenatal MeHg exposure in humans (Boucher et al., 2012; Grandjean et al., 1997), this has not been shown after postnatal exposure in either humans (Boucher et al., 2012) or rodents (Kendricks, Boomhower, & Newland, 2020; Kendricks & Newland, 2021). Rats exposed to MeHg during the ten days of neonatal development were similarly insensitive suggesting that exposure occurring after birth in rodents does not interact with processes of attention. This is substantiated by the fact that performance on the attention procedure in these rats was very similar to that seen in rats following adolescent exposure (Kendricks, Boomhower, Arnold, et al., 2020) suggesting similar processes are at play and that these processes are not being targeted by MeHg. However, it is also important to note that this may be related to the difference in exposure duration, as noted previously, such that a higher dose may be necessary to pull out these effects, if they exist.

Memory in the current procedure was not disrupted by MeHg, a conclusion that is similarly drawn in previous reports using this procedure in both rats (Kendricks et al., 2020) and mice (Kendricks et al., 2021) following exposure during adolescence. However, this conclusion differs from the drastic shift in memory noted both by Tian and colleagues (2016) and Sakamoto and colleagues (2004) when exposure occurred from the neonatal period to the middle of adolescence. The doses of exposure that produced such severe impact on memory reported by Sakamoto and colleagues (2004) were extraordinarily high, upward of 8-14X the highest dose used in the current study. Tian and colleagues (2016), however, used a dose similar to the highest dose used in the current study, at 400 µg/kg, but exposure in this study occurred intraperitoneally throughout the neonatal, late postnatal, and early adolescent periods. It is important to also note the drastic differences in procedures used to assess memory across these studies. The current

study, an assessment of visuospatial memory, is a far departure from the spatial memory assessed using Morris Water maze used by Tian and colleagues (2016) or the passive avoidance task employed by Sakamoto and colleagues (2004). Therefore, the differences in impact of MeHg on memory observed in this study may be linked to either the time course of exposure, the route of exposure (likely both), or the behavioral procedure used to assess the impact of MeHg on memory.

Neonatal Methylmercury on DAT

The degree of deficit observed by Tian and colleagues (2016) may be associated with damage to the developing hippocampus following exposure to MeHg. Tian and colleagues observed a dramatic reduction in newly developing cells in the dentate gyrus of rats after exposure to MeHg (Tian et al., 2016). This was further substantiated by a loss of neural stem cells in hippocampal tissue, *in vitro* (Tian et al., 2016). These changes suggest a role for MeHg in altering the trajectory of hippocampal maturation, a facet that is somewhat observed in the current study. Relatively little expression of DAT exists in the hippocampus (Guiard et al., 2008; Kwon et al., 2008), with expression possibly resulting from dopamine neurons from the ventral tegmental area innervating regions of the hippocampus (Guiard et al., 2008; Lisman & Grace, 2005). This reduced expression of DAT is similarly reported here with relatively little DAT observed in the hippocampus. Hippocampal DAT did tend to slightly vary by MeHg with marginally less DAT observed in the hippocampus for those rats exposed to 30 ppm MeHg. There were no such trends observed in either the striatum or the PFC for either 91-day-old rats or 8-month-old rats after behavioral testing. While the decrease in hippocampal DAT observed was not significant it suggests that the hippocampus may be relatively sensitive to early postnatal MeHg toxicity.

Limitations and Conclusions

Exposure during the neonatal period produces mild changes in behavior and neurochemistry, seen by increased perseverative behavior, impaired task acquisition, alterations in choice latency, and a mild, but not significant, shift in expression of DAT in the hippocampus in those animals exposed to MeHg.

While dopamine is a major player in hippocampal function, it is likely the case that MeHg may have exerted a more pronounced effect on increasing dopamine release into the hippocampus, a trend observed in the striatum (Faro et al., 1997, 1998), or by blocking activity of the norepinephrine transporter which is shown to clear hippocampal dopamine (Borgkvist et al., 2012). However, the impact of MeHg on the norepinephrine transporter is unlikely given the persistent lack of sensitivity of MeHg exposed animals to norepinephrine reuptake inhibitors (Kendricks & Newland, 2021; Reed & Newland, 2009). Given this, it may be necessary to observe changes in monoamine concentrations in these regions to assess better the impact of MeHg on neurotransmission.

Behavior in these animals was moderately impacted in a way that was akin to previous reports of exposure occurring during other developmental periods. However, these effects were subtle. It may be the case that the window of time that is encompassed by the neonatal period may be too small for long-term changes in neurochemistry and behavior to arise. This is unlike with the large changes noted by Tian and colleagues (2016), and the more drastic changes following very high doses of exposure noted by Sakamoto and colleagues (2004), who used longer windows of exposure. However, the fact that deficits in behavior still arose following exposure during this narrow window may suggest an alternative conclusion. If a higher dose of exposure were to be used, one that would allow for a more direct correlation with the degree of

toxicity seen following longer exposures, a more severe change in behavior may arise. In this way, it may be that the unique sensitivity of the neonatal period is more than what is showcased in the current study, an aspect of MeHg toxicity that needs to be further explored.

References

Barros, D. M., Ramirez, M. R., & Izquierdo, I. (2005). Modulation of working, short- and long-term memory by nicotinic receptors in the basolateral amygdala in rats. *Neurobiology of Learning and Memory*, *83*(2), 113–118. https://doi.org/10.1016/j.nlm.2004.10.001

Belém-Filho, I. J. A., Ribera, P. C., Nascimento, A. L., Gomes, A. R. Q., Lima, R. R., Crespo-Lopez, M. E., Monteiro, M. C., Fontes-Júnior, E. A., Lima, M. O., & Maia, C. S. F. (2018). Low doses of methylmercury intoxication solely or associated to ethanol binge drinking induce psychiatric-like disorders in adolescent female rats. *Environmental Toxicology and Pharmacology*, *60*, 184–194. https://doi.org/10.1016/j.etap.2018.04.021

Beyrouty, P., Stamler, C. J., Liu, J. N., Loua, K. M., Kubow, S., & Chan, H. M. (2006). Effects of prenatal methylmercury exposure on brain monoamine oxidase activity and neurobehaviour of rats. *Neurotoxicology and Teratology*, *28*(2), 251–259. https://doi.org/10.1016/j.ntt.2005.12.007

Boomhower, S. R., & Newland, M. C. (2017). Effects of adolescent exposure to methylmercury and d-amphetamine on reversal learning and an extradimensional shift in male mice. *Experimental and Clinical Psychopharmacology*, *25*(2), 64–73. https://doi.org/10.1037/pha0000107

Boomhower, S. R., & Newland, M. C. (2019a). Adolescent methylmercury exposure: Behavioral mechanisms and effects of sodium butyrate in mice. *NeuroToxicology*, *70*, 33–40. https://doi.org/10.1016/j.neuro.2018.10.011

Boomhower, S. R., & Newland, M. C. (2019b). d-Amphetamine and methylmercury exposure during adolescence alters sensitivity to monoamine uptake inhibitors in adult mice. *NeuroToxicology*, *72*, 61–73. https://doi.org/10.1016/j.neuro.2019.02.001

Borgkvist, A., Malmlöf, T., Feltmann, K., Lindskog, M., & Schilström, B. (2012). Dopamine in the hippocampus is cleared by the norepinephrine transporter. *International Journal of Neuropsychopharmacology*, *15*(4), 531–540. https://doi.org/10.1017/S1461145711000812

Boucher, O., Jacobson, S. W., Plusquellec, P., Dewailly, É., Ayotte, P., Forget-Dubois, N., Jacobson, J. L., & Muckle, G. (2012). Prenatal methylmercury, postnatal lead exposure, and evidence of attention deficit/hyperactivity disorder among Inuit children in Arctic Québec. *Environmental Health Perspectives*, *120*(10), 1456–1461. https://doi.org/10.1289/ehp.1204976

Burger, J., Gochfeld, M., Jeitner, C., Donio, M., & Pittfield, T. (2012). Interspecific and intraspecific variation in selenium:mercury molar ratios in saltwater fish from the Aleutians: Potential protection on mercury toxicity by selenium. *Science of the Total Environment*, *431*, 46–56. https://doi.org/10.1016/j.scitotenv.2012.05.024

Burgos, H., Mardones, L., Campos, M., Castillo, A., Fernández, V., & Hernández, A. (2005). Chronic treatment with clomipramine and desipramine induces deficit in long-term visuo-spatial memory of rats. *International Journal of Neuroscience*, *115*(1), 47–54. https://doi.org/10.1080/00207450490512641

Bushnell, P. J., & Levin, E. D. (1993). Effects of dopaminergic drugs on working and reference memory in rats. *Pharmacology, Biochemistry and Behavior*, *45*(4), 765–776. https://doi.org/10.1016/0091-3057(93)90119-E

Bushnell, P. J., Oshiro, W. M., & Padnos, B. K. (1997). Detection of visual signals by rats: Effects of chlordiazepoxide and cholinergic and adrenergic drugs on sustained attention. *Psychopharmacology*, *134*(3), 230–241. https://doi.org/10.1007/s002130050446

Castoldi, A. F., Blandini, F., Randine, G., Samuele, A., Manzo, L., & Coccini, T. (2006). Brain monoaminergic neurotransmission parameters in weanling rats after perinatal exposure to methylmercury and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB153). *Brain Research, 1112*(1), 91–98. https://doi.org/10.1016/j.brainres.2006.07.022

Cheon, K. A., Ryu, Y. H., Kim, Y. K., Namkoong, K., Kim, C. H., & Lee, J. D. (2003). Dopamine transporter density in the basal ganglia assessed with [123 I]IPT SPET in children with attention deficit hyperactivity disorder. *European Journal of Nuclear Medicine and Molecular Imaging*, *30*(2), 306–311. https://doi.org/10.1007/s00259-002-1047-3

Cherian, A. K., Tronson, N. C., Parikh, V., Kucinski, A., Blakely, R. D., & Sarter, M. (2019). Repetitive mild concussion in subjects with a vulnerable cholinergic system: Lasting cholinergic-attentional impairments in CHT+/- mice. *Behavioral Neuroscience*, *133*(4), 448–459. https://doi.org/10.1037/bne0000310

Cheuk, D. K. L., & Wong, V. (2006). Attention-deficit hyperactivity disorder and blood mercury level: A case-control study in Chinese children. *Neuropediatrics*, *37*(4), 234–240. https://doi.org/10.1055/s-2006-924577

Clarke, H. F., Dalley, J. W., Crofts, H. S., Robbins, T. W., & Roberts, A. C. (2004). Cognitive Inflexibility after Prefrontal Serotonin Depletion. *Science*, *304*(5672), 878–880. https://doi.org/10.1126/science.1094987

Cools, R., Frank, M. J., Gibbs, S. E., Miyakawa, A., Jagust, W., & D'Esposito, M. (2009). Striatal dopamine predicts outcome-specific reversal learning and its sensitivity to dopaminergic drug administration. *Journal of Neuroscience*, *29*(5), 1538–1543. https://doi.org/10.1523/JNEUROSCI.4467-08.2009

Cordier, S., Garel, M., Mandereau, L., Morcel, H., Doineau, P., Gosme-Seguret, S., Josse, D., White, R., & Amiel-Tison, C. (2002). Neurodevelopmental investigations among methylmercury-exposed children in French Guiana. *Environmental Research*, *89*(1), 1–11. https://doi.org/10.1006/enrs.2002.4349

Davidson, P. W., Cory-Slechta, D. A., Thurston, S. W., Huang, L. S., Shamlaye, C. F., Gunzler, D., Watson, G., van Wijngaarden, E., Zareba, G., Klein, J. D., Clarkson, T. W., Strain, J. J., & Myers, G. J. (2011). Fish consumption and prenatal methylmercury exposure: Cognitive and behavioral outcomes in the main cohort at 17 years from the Seychelles child development study. *NeuroToxicology*, *32*(6), 711–717. https://doi.org/10.1016/j.neuro.2011.08.003

Davidson, P. W., Myers, G. J., Cox, C., Axtell, C., Shamlaye, C., Sloane-reeves, J., Cernichiari, E., Needham, L., Choi, A., Wang, Y., Berlin, M., & Clarkson, T. W. (1998). Effects of prenatal and postnatal methylmecury exposure from fish consumption on neurodevelopment: Outcomes at 66 months of age in the Seychelles child development study. *JAMA*, *280*(8), 701–707.

Debes, F., Budtz-Jørgensen, E., Weihe, P., White, R. F., & Grandjean, P. (2006). Impact of prenatal methylmercury exposure on neurobehavioral function at age 14 years. *Neurotoxicology and Teratology*, *28*(5), 536–547. https://doi.org/10.1016/j.ntt.2006.02.005

Dreiem, A., Shan, M., Okoniewski, R. J., Sanchez-Morrissey, S., & Seegal, R. F. (2009). Methylmercury inhibits dopaminergic function in rat pup synaptosomes in an age-dependent manner. *Neurotoxicology and Teratology*, *31*(5), 312–317. https://doi.org/10.1016/j.ntt.2009.05.001

Duda, W., Wesierska, M., Ostaszewski, P., Vales, K., Nekovarova, T., & Stuchlik, A. (2016). MK-801 and memantine act differently on short-term memory tested with different time-intervals in the Morris water maze test. *Behavioural Brain Research*, *311*, 15–23. https://doi.org/10.1016/j.bbr.2016.05.024

Faro, L. R. F., do Nascimento, J. L. M., Alfonso, M., & Durán, R. (1998). Acute administration of methylmercury changes in vivo dopamine release from rat striatum. *Bulletin of Environmental Contamination and Toxicology*, *60*(4), 632–638. https://doi.org/10.1007/s001289900673

Faro, L. R. F., do Nascimento, J. L. M., Alfonso, M., & Durán, R. (2002). Mechanism of action of methylmercury on in vivo striatal dopamine release: Possible involvement of dopamine transporter. *Neurochemistry International*, *40*(5), 455–465. https://doi.org/10.1016/S0197-0186(01)00098-5

Faro, L. R. F., Durán, R., do Nascimento, J. L. M., Alfonso, M., & Picanço-Diniz, C. W. (1997). Effects of methyl mercury on the in vivo release of dopamine and its acidic metabolites DOPAC and HVA from striatum of rats. *Ecotoxicology and Environmental Safety*, *38*(2), 95–98. https://doi.org/10.1006/eesa.1997.1567

Fréry, N., Maury-Brachet, R., Maillot, E., Deheeger, M., De Mérona, B., & Boudou, A. (2001). Gold-mining activities and mercury contamination of native Amerindian communities in French Guiana: Key role of fish in dietary uptake. *Environmental Health Perspectives*, *109*(5), 449–456. https://doi.org/10.1289/ehp.01109449

Giménez-Llort, L., Ahlbom, E., Daré, E., Vahter, M., Ögren, S. O., & Ceccatelli, S. (2001). Prenatal exposure to methylmercury changes dopamine-modulated motor activity during

early ontogeny: Age and gender-dependent effects. *Environmental Toxicology and Pharmacology*, *9*(3), 61–70. https://doi.org/10.1016/S1382-6689(00)00060-0

Grandjean, P., Weihe, P., Debes, F., Choi, A. L., & Budtz-Jørgensen, E. (2014). Neurotoxicity from prenatal and postnatal exposure to methylmercury. *Neurotoxicology and Teratology*, *43*, 39–44. https://doi.org/10.1016/j.ntt.2014.03.004

Grandjean, P., Weihe, P., Jørgensen, P. J., Clarkson, T., Cernichiari, E., & Viderø, T. (1992). Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. In *Archives of environmental health* (Vol. 47, Issue 3, pp. 185–195). https://doi.org/10.1080/00039896.1992.9938348

Grandjean, P., Weihe, P., White, R. F., & Debes, F. (1998). Cognitive performance of children prenatally exposed to "safe" levels of methylmercury. *Environmental Research*, *77*(2), 165–172. https://doi.org/10.1006/enrs.1997.3804

Grandjean, P., Weihe, P., White, R. F., Debes, F., Araki, S., Yokoyama, K., Murata, K., SØrensen, N., Dahl, R., & JØrgensen, P. J. (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicology and Teratology*, *19*(6), 417–428. https://doi.org/10.1016/S0892-0362(97)00097-4

Guiard, B. P., el Mansari, M., & Blier, P. (2008). Cross-talk between dopaminergic and noradrenergic systems in the rat ventral tegmental area, locus ceruleus, and dorsal hippocampus. *Molecular Pharmacology*, *74*(5), 1463–1475. https://doi.org/10.1124/mol.108.048033

Ha, M., Kwon, H.-J., Lim, M.-H., Jee, Y.-K., Hong, Y.-C., Leem, J.-H., Sakong, J., Bae, J.-M., Hong, S.-J., Roh, Y.-M., & Jo, S.-J. (2009). Low blood levels of lead and mercury and symptoms of attention deficit hyperactivity in children: A report of the children's health

and environment research (CHEER). *NeuroToxicology*, *30*, 31–36. https://doi.org/10.1016/j.neuro.2008.11.011

Kehagia, A. A., Murray, G. K., & Robbins, T. W. (2010). Learning and cognitive flexibility: Frontostriatal function and monoaminergic modulation. *Current Opinion in Neurobiology*, *20*(2), 199–204. https://doi.org/10.1016/j.conb.2010.01.007

Kendricks, D. R., Boomhower, S. R., Arnold, M. A., Glenn, D. J., & Newland, M. C. (2020). Adolescent methylmercury exposure alters short-term remembering, but not sustained attention, in male Long-Evans rats. *NeuroToxicology*, *78*, 186–194. https://doi.org/10.1016/j/neuro.2020.03.009

Kendricks, D. R., Boomhower, S. R., & Newland, M. C. (2020). Methylmercury, attention, and memory: Baseline-dependent effects of adult d-amphetamine and marginal effects of adolescent methylmercury. *NeuroToxicology*, *80*, 130–139. https://doi.org/10.1016/j.neuro.2020.07.009

Kendricks, D. R., & Newland, M. C. (2021). Selective dopaminergic effects on attention and memory in male mice exposed to Methylmercury during adolescence. *Neurotoxicology and Teratology*, *87*, 107016. https://doi.org/10.1016/j.ntt.2021.107016

Kim, S., Arora, M., Fernandez, C., Landero, J., Caruso, J., & Chen, A. (2013). Lead, mercury, and cadmium exposure and attention deficit hyperactivity disorder in children. *Environmental Research*, *126*, 105–110. https://doi.org/10.1016/j.envres.2013.08.008

Komulainen, H., & Tuomisto, J. (1981). Interference of Methyl Mercury with Monoamine Uptake and Release in Rat Brain Synaptosomes. *Acta Pharmacologica et Toxicologica*, *48*(3), 214–222. https://doi.org/10.1111/j.1600-0773.1981.tb01611.x

Kucinski, A., Phillips, K. B., Cherian, A. K., & Sarter, M. (2020). Rescuing the attentional performance of rats with cholinergic losses by the M1 positive allosteric modulator TAK-071. *Psychopharmacology*, *237*(1), 137–153. https://doi.org/10.1007/s00213-019-05354-5

Kwon, O. bin, Paredes, D., Gonzalez, C. M., Org Neddens, J. ¨, Hernandez, L., Vullhorst, D., & Buonanno, A. (2008). Neuregulin-1 regulates LTP at CA1 hippocampal synapses through activation of dopamine D4 receptors. In *PNAS October* (Vol. 7, Issue 40).

Lisman, J. E., & Grace, A. A. (2005). The hippocampal-VTA loop: Controlling the entry of information into long-term memory. In *Neuron* (Vol. 46, Issue 5, pp. 703–713). https://doi.org/10.1016/j.neuron.2005.05.002

Mohler, E. G., Heck, W. H., & Williams, C. L. (2001). Sustained attention in adult mice is modulated by prenatal choline availability. *International Journal of Comparative Psychology*, *14*(3), 136–150. http://escholarship.org/uc/item/2d4086s1

Myers, G. J., Davidson, P. W., Cox, C., Shamlaye, C. F., Palumbo, D., Cernichiari, E., Sloane-reeves, J., Wilding, G. E., Kost, J., Huang, L. S., & Clarkson, T. W. (2003). Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study. *Lancet*, *361*(9370), 1686–1692.

Newland, M. C., Hoffman, D. J., Heath, J. C., & Donlin, W. D. (2013). Response inhibition is impaired by developmental methylmercury exposure: Acquisition of low-rate lever-pressing. *Behavioural Brain Research*, *253*, 196–205. https://doi.org/10.1016/j.bbr.2013.05.038

Newland, M. C., Paletz, E. M., & Reed, M. N. (2008). Methylmercury and nutrition: Adult effects of fetal exposure in experimental models. *NeuroToxicology*, *29*(5), 783–801. https://doi.org/10.1016/j.neuro.2008.06.007

Newland, M. C., & Reile, P. A. (1999). Blood and brain mercury levels after chronic gestational exposure to methylmercury in rats. *Toxicological Sciences*, *50*(1), 106–116. https://doi.org/10.1093/toxsci/50.1.106

Newland, M. C., Reile, P. A., & Langston, J. L. (2004). Gestational exposure to methylmercury retards choice in transition in aging rats. *Neurotoxicology and Teratology*, *26*(2), 179–194. https://doi.org/10.1016/j.ntt.2003.12.004

Newland, M. C., Yezhou, S., Lögdberg, B., & Berlin, M. (1994). Prolonged behavioral effects of in utero exposure to lead or methyl mercury: Reduced sensitivity to changes in reinforcement contingencies during behavioral transitions and in steady state. *Toxicology and Applied Pharmacology*, *126*, 6–15. https://doi.org/10.1006/taap.1994.1084

Oliveira, A. N., Pinheiro, A. M., Belém-Filho, I. J. A., Fernandes, L. M. P., Cartágenes, S. C., Ribera, P. C., Fontes-Júnior, E. A., Crespo-Lopez, M. E., Monteiro, M. C., Lima, M. O., & Maia, C. S. F. (2018). Unravelling motor behaviour hallmarks in intoxicated adolescents: methylmercury subtoxic-dose exposure and binge ethanol intake paradigm in rats. *Environmental Science and Pollution Research*, *25*(22), 21937–21948. https://doi.org/10.1007/s11356-018-2235-4

Onishchenko, N., Tamm, C., Vahter, M., Hökfelt, T., Johnson, J. A., Johnson, D. A., & Ceccatelli, S. (2007). Developmental exposure to methylmercury alters learning and induces depression-like behavior in male mice. *Toxicological Sciences*, *97*(2), 428–437. https://doi.org/10.1093/toxsci/kfl199

Overman, W. H. (2004). Sex differences in early childhood, adolescence, and adulthood on cognitive tasks that rely on orbital prefrontal cortex. *Brain and Cognition*, *55*(1), 134–147. https://doi.org/10.1016/S0278-2626(03)00279-3

Paletz, E. M., Day, J. J., Craig-Schmidt, M. C., & Newland, M. C. (2007). Spatial and visual discrimination reversals in adult and geriatric rats exposed during gestation to methylmercury and n - 3 polyunsaturated fatty acids. *NeuroToxicology*, *28*(4), 707–719. https://doi.org/10.1016/j.neuro.2007.05.001

Piotrowski, J. K., & Buchanan, J. M. (1982). Dose-Time-Response Functions for Toxic Chemicals. *Environmental Monitoring and Assessment*, *2*, 139–156.

Pope, D. A., Boomhower, S. R., Hutsell, B. A., Teixeira, K. M., & Newland, M. C. (2016). Chronic cocaine exposure in adolescence: Effects on spatial discrimination reversal, delay discounting, and performance on fixed-ratio schedules in mice. *Neurobiology of Learning and Memory*, *130*, 93–104. https://doi.org/10.1016/j.nlm.2016.01.017

Rasmussen, E. B., & Newland, M. C. (2001). Developmental exposure to methylmercury alters behavioral sensitivity to d-amphetamine and pentobarbital in adult rats. *Neurotoxicology and Teratology*, *23*(1), 45–55. https://doi.org/10.1016/S0892-0362(00)00112-4

Reed, M. N., & Newland, M. C. (2009). Gestational methylmercury exposure selectively increases the sensitivity of operant behavior to cocaine. *Behavioral Neuroscience*, *123*(2), 408–417.

Reed, M. N., Paletz, E. M., & Newland, M. C. (2006). Gestational exposure to methylmercury and selenium: Effects on a spatial discrimination reversal in adulthood. *NeuroToxicology*, *27*, 721–732. https://doi.org/10.1016/j.neuro.2006.03.022

Rezvani, A. H., Bushnell, P. J., & Levin, E. D. (2002). Effects of nicotine and mecamylamine on choice accuracy in an operant visual signal detection task in female rats. *Psychopharmacology*, *164*(4), 369–375. https://doi.org/10.1007/s00213-002-1221-0

Sagvolden, T., & Xu, T. (2008). l-Amphetamine improves poor sustained attention while d-amphetamine reduces overactivity and impulsiveness as well as improves sustained attention in an animal model of attention-deficit/hyperactivity disorder (ADHD). *Behavioral and Brain Functions : BBF*, *4*, 3. https://doi.org/10.1186/1744-9081-4-3

Sakamoto, M., Kakita, A., Bezerra De Oliveira, R., Sheng Pan, H., & Takahashi, H. (2004). Dose-dependent effects of methylmercury administered during neonatal brain spurt in rats. *Developmental Brain Research*, *152*(2), 171–176. https://doi.org/10.1016/j.devbrainres.2004.06.016

Sakamoto, M., Kakita, A., Wakabayashi, K., Takahashi, H., Nakano, A., & Akagi, H. (2002). Evaluation of changes in methylmercury accumulation in the developing rat brain and its effects: A study with consecutive and moderate dose exposure throughout gestation and lactation periods. *Brain Research*, *949*(1–2), 51–59. https://doi.org/10.1016/S0006-8993(02)02964-5

Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., & Nobel-Haeusslein, L. J. (2013). Brain development in rodent and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Progress in Neurobiology*, *106–107*, 1–16. https://doi.org/10.1016/j.pneurobio.2013.04.001

Strain, J. J., Davidson, P. W., Bonham, M. P., Duffy, E. M., Stokes-Riner, A., Thurston, S. W., Wallace, J. M. W., Robson, P. J., Shamlaye, C. F., Georger, L. A., Sloane-Reeves, J., Cernichiari, E., Canfield, R. L., Cox, C., Huang, L. S., Janciuras, J., Myers, G. J., &

Clarkson, T. W. (2008). Associations of maternal long-chain polyunsaturated fatty acids, methyl mercury, and infant development in the Seychelles Child Development Nutrition Study. *NeuroToxicology*, *29*(5), 776–782. https://doi.org/10.1016/j.neuro.2008.06.002

Tian, J., Luo, Y., Chen, W., Yang, S., Wang, H., Cui, J., Lu, Z., Lin, Y., & Bi, Y. (2016). MeHg Suppressed Neuronal Potency of Hippocampal NSCs Contributing to the Puberal Spatial Memory Deficits. *Biological Trace Element Research*, *172*(2), 424–436. https://doi.org/10.1007/s12011-015-0609-8

Turchi, J., & Sarter, M. (2001). Bidirectional modulation of basal forebrain N-Methyl-D-Aspartate receptor function differentially affects visual attention but not visual discrimination performance. *Neuroscience*, *104*(2), 407–417. www.elsevier.com/locate/neuroscience

van Wijngaarden, E., Thurston, S. W., Myers, G. J., Harrington, D., Cory-Slechta, D. A., Strain, J. J., Watson, G. E., Zareba, G., Love, T., Henderson, J., Shamlaye, C. F., & Davidson, P. W. (2017). Methyl mercury exposure and neurodevelopmental outcomes in the Seychelles Child Development Study Main cohort at age 22 and 24 years. *Neurotoxicology and Teratology*, *59*, 35–42. https://doi.org/10.1016/j.ntt.2016.10.011

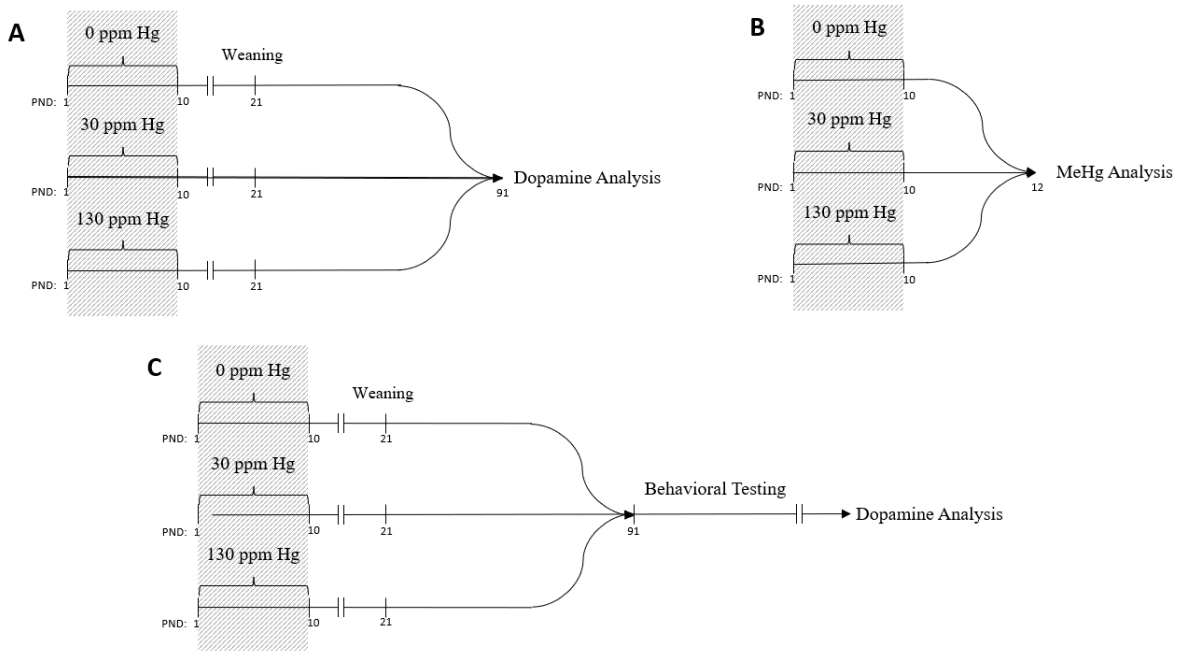


Figure 1: Exposure period for animals undergoing behavioral testing, dopamine analyses, and MeHg assessment. A) 19 animals were euthanized on PND 91 for dopamine analyses. These animals were exposed to MeHg from PND 1-10, weaned on PND 21, and euthanized on PND 91. Brains were harvested and DAT concentrations were assessed via western blot. B) 19 animals (N = 7 cohort 1; N = 12 cohort 2) were used for MeHg assessment. These animals were exposed to MeHg from PND 1-10 and euthanized on either PND 12 (N = 14) or PND 13 (N = 3). Brains were removed and MeHg content was assessed using ICP-MS. C) 36 animals underwent behavioral testing. These animals were exposed to MeHg from PND 1-10, weaned on PND 21, and behavioral testing began on PND 91. After behavioral testing, DAT concentration was assessed in 18 of these animals.

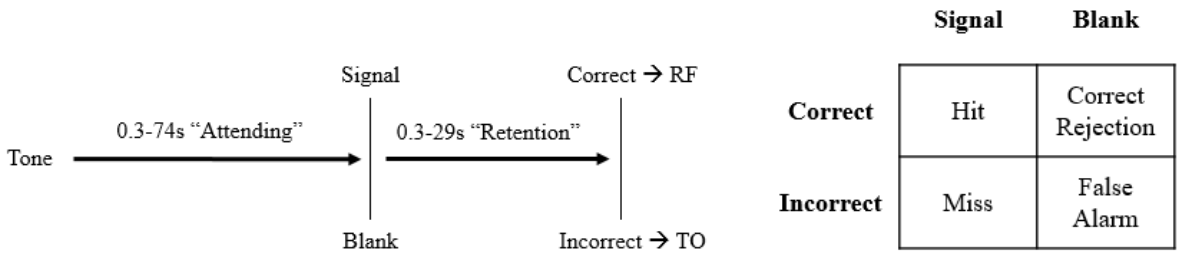


Figure 2: Schematic of the attention procedure. Animals were required to accurately discriminate between the presence and absence of a 0.3s visual signal. Accurate detection of the signal ("Hit") and accurate rejection of the signal ("Correct Rejection") resulted in sucrose reinforcement (RF). Inaccurate detection of the signal ("Miss") and inaccurate rejection of the signal ("False Alarm") resulted in a 3s timeout (TO).

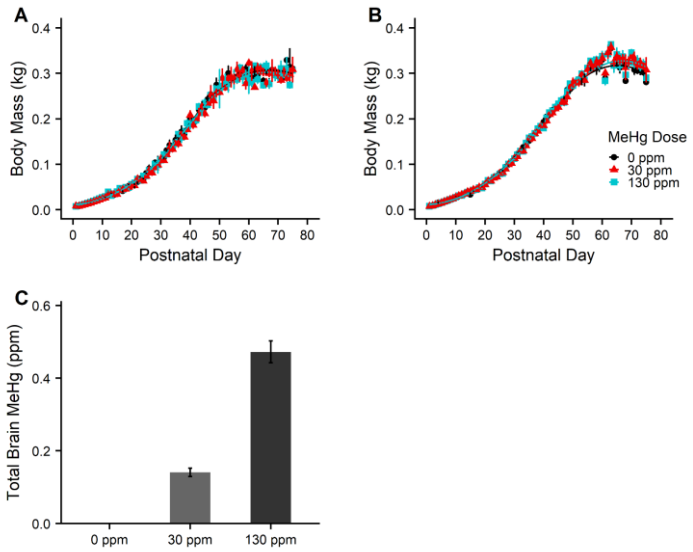


Figure 3: Body mass from PND 1-75 for A) animals euthanized on PND 91 for dopamine analysis and B) animals that began behavioral training on PND 91. C) Whole brain MeHg concentration per exposure group. There were no significant differences in body mass. Brain MeHg concentration in 0ppm animals was below detectable limits. There were clear difference in brain Hg concentration between the 30 and 130ppm groups but these values are lower than those seen in previous studies of MeHg exposure. Lines in A and B are LOESS, colored markers in B represent MeHg groups for A and B. Values are means ± SEM.

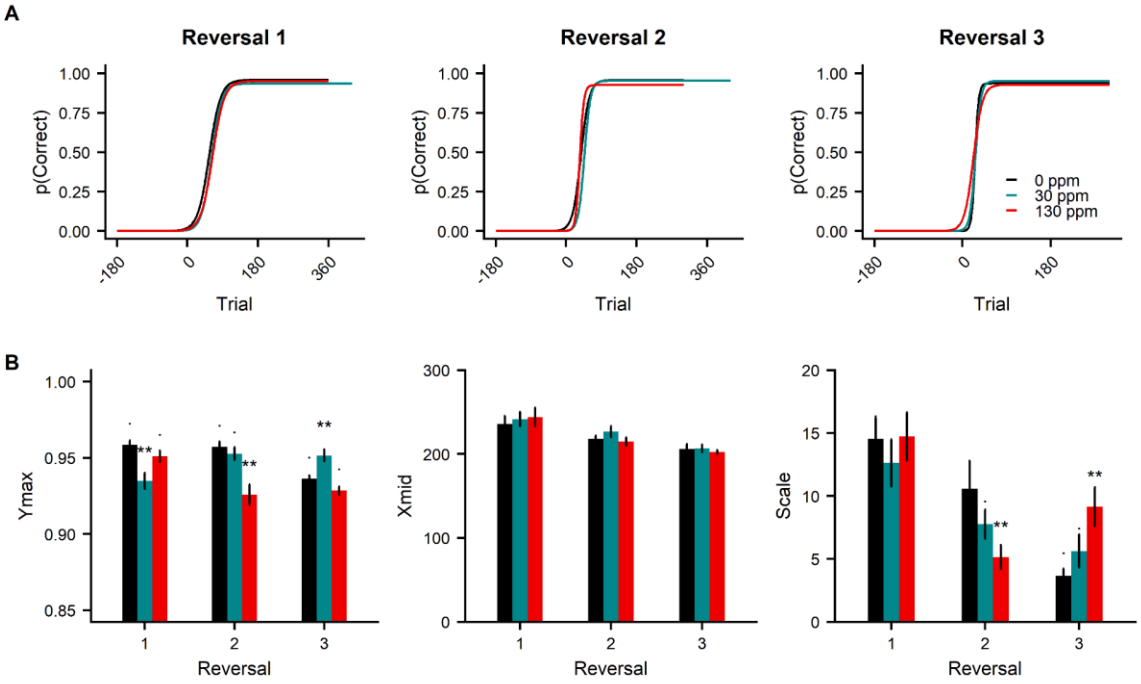


Figure 4: Neonatal MeHg on transitions at A) Reversal 1 (R1), Reversal 2 (R2), and Reversal 3 (R3). Small MeHg-related differences were observed for Y_{max} in R1 and R2 and for scale in R2 and R3. Lines are the model fit from Eq. 1. Coefficients were isolated for Y_{max}, Xmid, and scale at each reversal. B) Y_{max} was MeHg-dependent with lower Y_{max} achieved in 30 ppm animals in R1 and higher in R3. In R2, animals exposed to 130 ppm achieved lower Y_{max}. Xmid did not vary by MeHg but scale was lower for animals exposed to 130 ppm in R2 and higher for these animals in R3. In B, "**" are statistically different ($p < 0.05$) from other groups. Colored markers represent MeHg exposure groups.

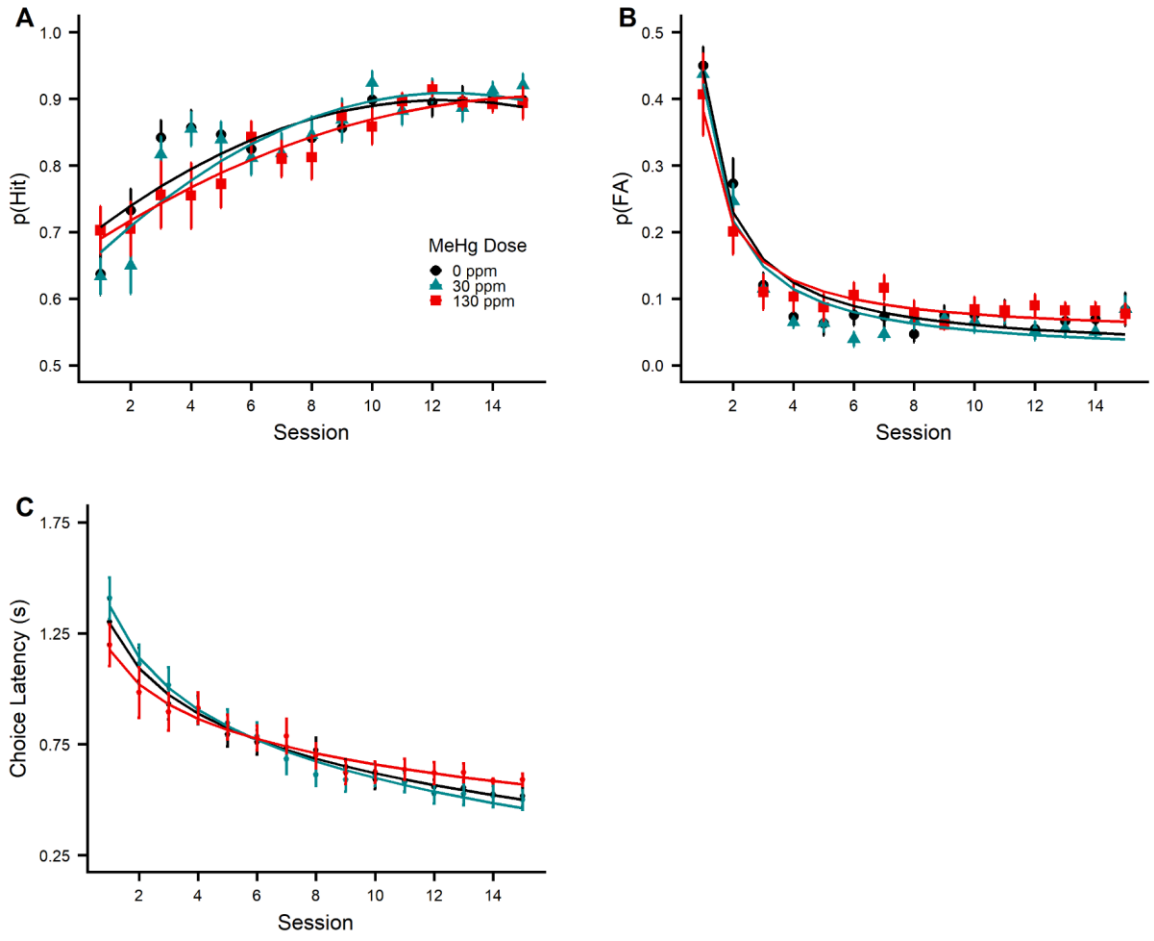


Figure 5: Accuracy and choice latency during acquisition. A) Accurate responding under the signal, p(Hit), increased during training while B) inaccurate responding in the signal's absence, p(FA), was reduced. MeHg impaired the acquisition of low rates of incorrect responses in the signal's absence, p(FA). C) Choice latency reliably decreased throughout training with the slope of this decrease being dependent on MeHg. Lines are model fits. Values are means ± SEM.

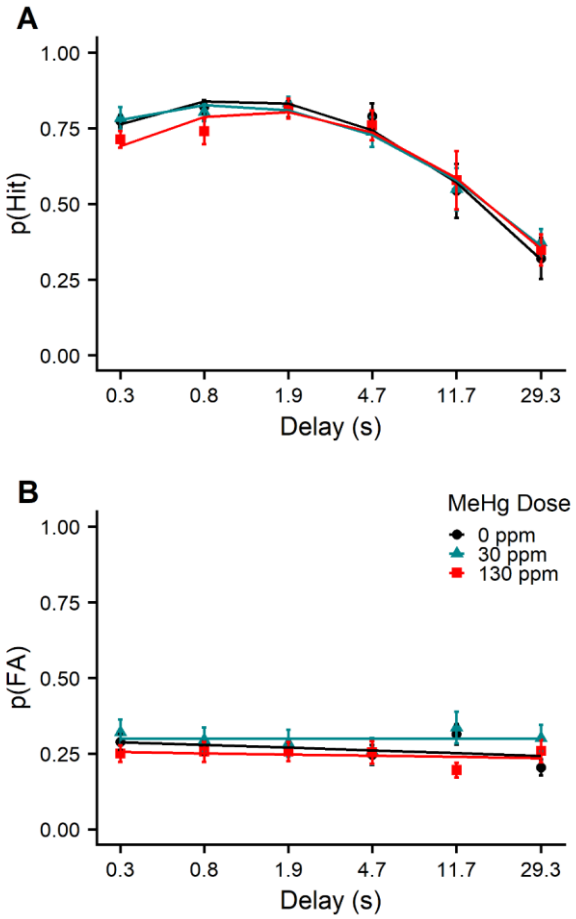


Figure 6: Accuracy across retention delays during the memory condition of sustained attention. A) p(Hit) across retention delays was modeled using a quadratic function. B) p(FA) across these delays was modeled using a linear function. p(Hit) varied by delay, but p(FA) did not. Neither was dependent on MeHg. Values are means ± SEM. Colored markers in B represent MeHg exposure groups for A and B.

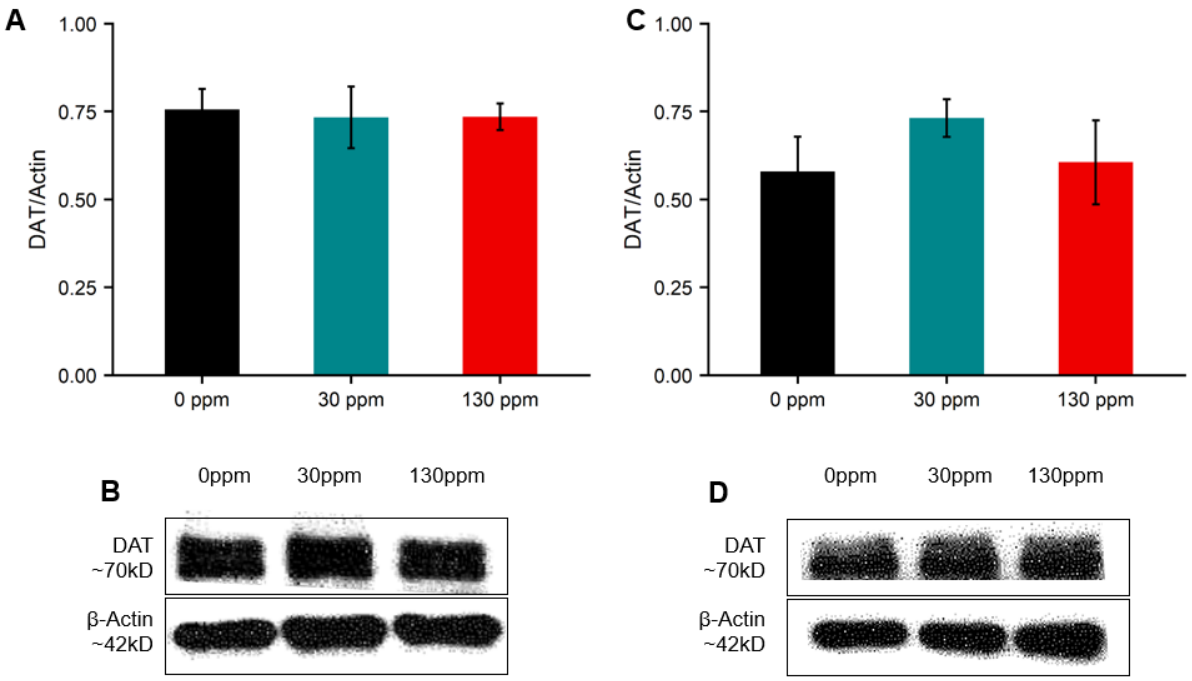


Figure 7: DAT in PND 91 animals, expressed as a proportion of the loading control, β-Actin. DAT expression in the striatum (A) and representative bands (B) at approximately 70kD for DAT and 42kD for β-actin. DAT expression in the PFC (C) and representative bands (D) in animals from each exposure group. Values in A and C are means ± SEM.

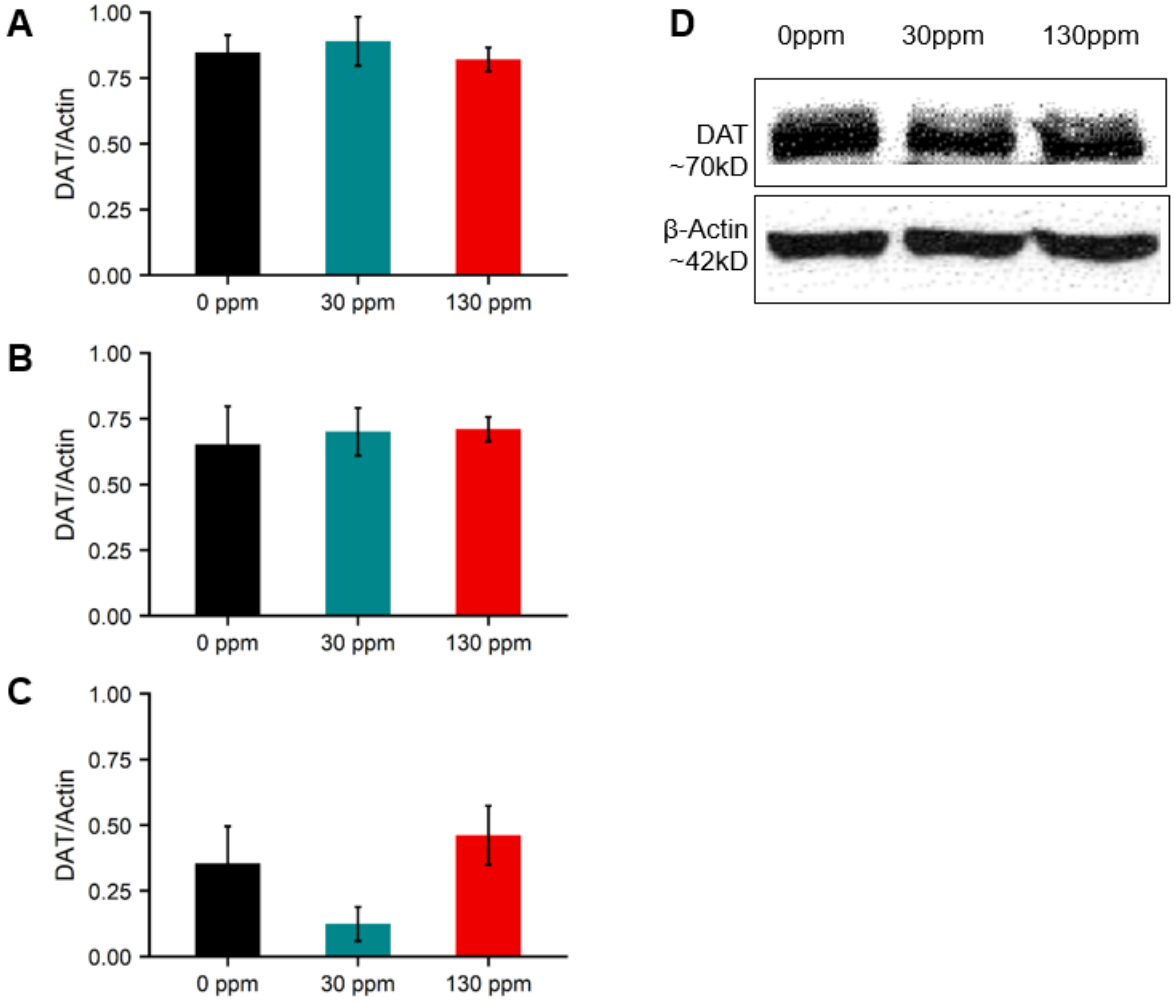
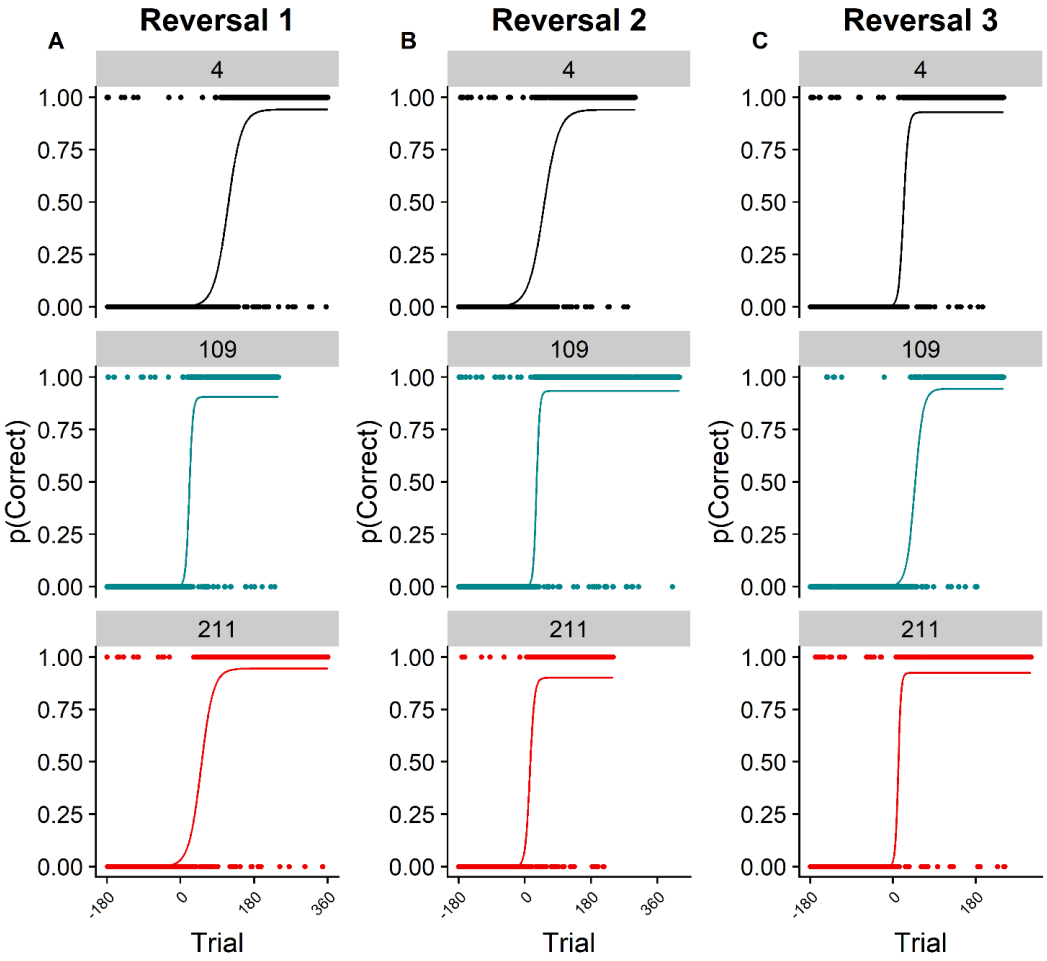
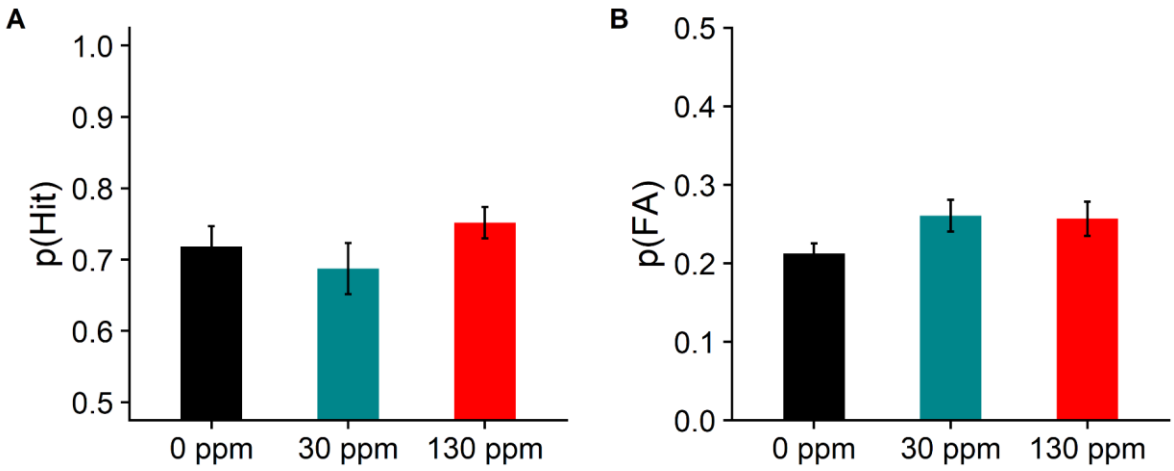


Figure 8: DAT in 8-month-old animals after behavioral testing. DAT expression in the striatum (A), prefrontal cortex (B), and hippocampus (C) as expressed as a proportion of the loading control, β-actin. (D) Representative bands from the PFC are shown for DAT at approximately 70kD and β-actin at 42kD. Values in A-C are means ± SEM.

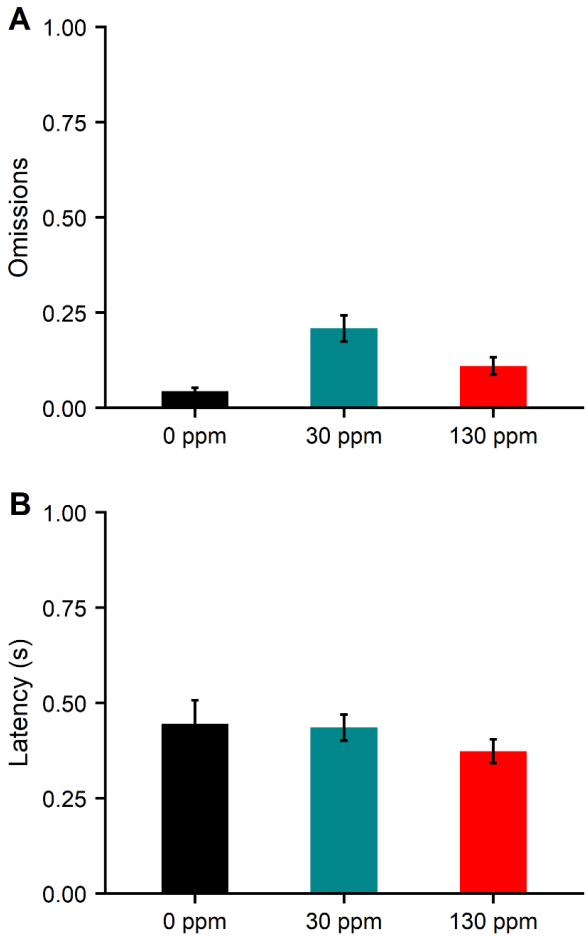
Appendix: Supplementary Data



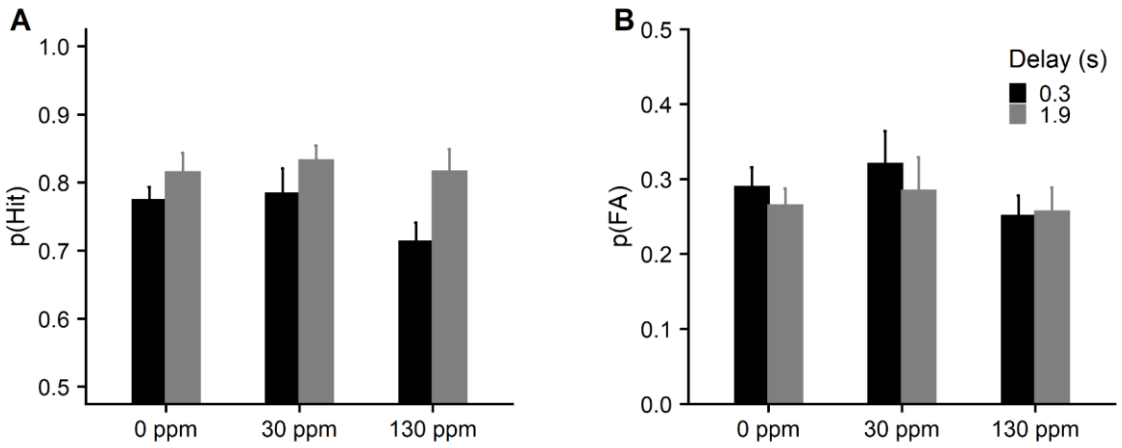
S-Figure 1: Representative individual subject data from SDR. Data are from three subjects, across three groups, for A) Reversal 1, B) Reversal 2, and C) Reversal 3. The x-axis shows the trial number with the trial labeled "0" being when the lever switched. Values before that are coded as "0" when accurate and values after are coded as "1" when accurate. Colors represents different exposure groups: control = black, 30 ppm = teal, and 130 ppm = red. Lines are model fits. Values are individual responses.



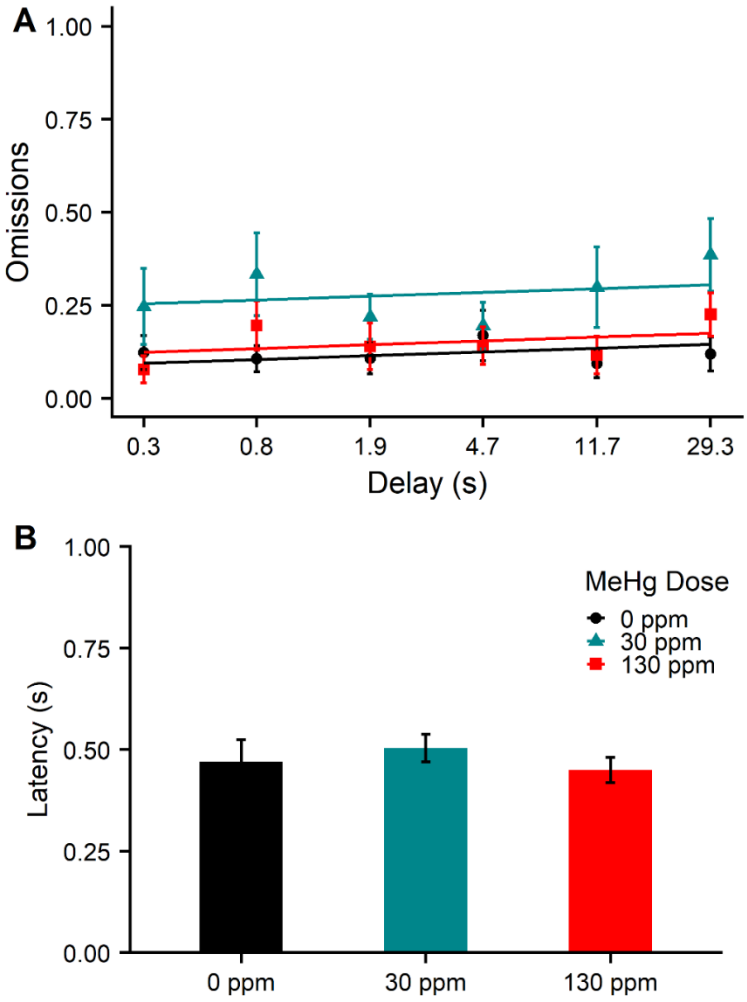
S-Figure 2: Accuracy during the baseline condition of sustained attention. Accuracy is modeled using A) Hit rate (p(Hit)), and B) false-alarm rate (p(FA)). There were no MeHg-related differences in p(Hit) or p(FA). Values are means ± SEM.



S-Figure 3: Total A) omissions and B) choice latency during the baseline condition of sustained attention. MeHg caused a slight increase in the number of omissions in animals exposed to 30 ppm MeHg. There was no effect of MeHg on choice latency. Values are means ± SEM.



S-Figure 4: Accuracy at the 0.3 and 1.9s delays. A) p(Hit) was higher at the 1.9s delay compared to the 0.3s delay, and this difference was significant. B) p(FA) did not differ across these delays. Neither p(Hit) nor p(FA) at these delays was dependent on MeHg. Values are means ± SEM. Colored markers in B designate the delay for A and B.



S-Figure 5: Total omissions and choice latency during the memory condition of sustained attention. A) Omissions slightly increased across retention delays, linearly. Animals exposed to 30 ppm MeHg had overall slightly higher omissions across all delays. B) Choice latency did not vary by MeHg. Values are means ± SEM. Colored markers in B represent MeHg exposure groups for A and B.